Rapid lipid enrichment in ω3 fatty acids: Liver data

YVON A. CARPENTIER, SEBASTIEN PELTIER, LAURENCE PORTOIS, ABDULLAH SENER and WILLY J. MALAISSE

Laboratories of Experimental Hormonology and Surgery, Brussels Free University, Brussels, Belgium

Received November 7, 2007; Accepted December 11, 2007

Abstract. The bolus intravenous injection of a novel medium-chain triglyceride:fish oil emulsion to normal subjects was recently reported to enrich within 60 min the phospholipid content of leucocytes and platelets in long-chain polyunsaturated ω3 fatty acids. The present study, conducted in second generation ω3-depleted rats, aims at investigating whether such a procedure may also increase within 60 min the phospholipid content of ω3 fatty acids in cells located outwards of the bloodstream, in this case liver cells, and whether this coincides with correction of the perturbation in the liver triglyceride fatty acid content and profile otherwise prevailing in these rats. The results indicate that such is indeed the case and further suggest a cause-to-effect relationship between the two events.

Introduction

In the first report in this series, the fatty acid pattern of plasma phospholipids, triglycerides, diglycerides and unesterified fatty acids was examined in rats depleted in long-chain polyunsaturated ω3 fatty acids and injected, or not, intravenously 60 min before sacrifice with either an ω3 fatty acid-rich middle-chain triglyceride:fish oil emulsion (MCT:FO) or a control ω3 fatty acid-poor middle-chain triglyceride:olive oil emulsion (MCT:OO) (1). The present report deals mainly with the changes observed, under these experimental conditions, in the fatty acid pattern of liver lipids in both male and female animals.

Materials and methods

The Materials and methods of the present study were already defined in the first report in this series (1).

Correspondence to: Professor W.J. Malaisse, Laboratory of Experimental Hormonology, Brussels Free University, 808 Route de Lennik, B-1070 Brussels, Belgium
E-mail: malaisse@ulb.ac.be

Key words: second generation rats depleted in long-chain polyunsaturated ω3 fatty acids, plasma and liver lipid fatty acid profile, medium-chain triglyceride:fish oil emulsion

Results

Liver phospholipids. The total fatty acid content of the liver phospholipids was close to 20 mg per g wet weight in the five groups of rats listed in Table I.

Sizeable amounts of C20:5ω3 in the liver phospholipids were only detected in the rats injected with the MCT:FO emulsion. In the male rats, the C22:5ω3 and C22:6ω3 content of phospholipids was also much higher ($p \leq 0.02$) in the rats injected with the MCT:FO emulsion than in the animals injected with the MCT:OO emulsion, in which the mean weight percentage of C22:6ω3 was already higher ($p<0.005$) than in the non-injected ω3-depleted rats. Likewise, the weight percentage of C22:5ω3 and C22:6ω3 in the phospholipids of female rats was increased ($p<0.025$) after injection of the MCT:FO emulsion, respectively from 0.14±0.02 and 1.74±0.05% in the control non-injected animals (n=2) to 0.35±0.03 and 2.36±0.10% in the female FO rats (n=3). The C22:5ω3/C22:6ω3 ratio in the liver phospholipids was higher ($p \leq 0.05$) in the rats injected with the MCT:FO emulsion than in the uninjected rats (or animals injected with the MCT:OO emulsion). As documented in Table I, gender differences were also on occasion encountered, as illustrated by the much higher C22:6ω3 content ($p<0.001$) and much lower C22:5ω3/C22:6ω3 ratio ($p<0.005$) in female versus male uninjected ω3-depleted rats.

Some differences were also encountered concerning the saturated or monosaturated middle-chain and long-chain fatty acids. First, sizeable amounts of C8:0 (11.4±1.0 μg/g; n=3) and C10:0 (18.1±2.4 μg/g; n=7) were only detected in some rats injected with either the MCT:OO or MCT:FO emulsion, whilst such was never the case in the uninjected animals. In this respect, the C8:0 and C10:0 content of phospholipids was obviously lower in male than female rats (Table I). In the latter rats, the C8:0/C10:0 ratio averaged 50.0±4.3% (n=3). The amount of C14:0 in the liver phospholipids was lower in female animals than in male rats and, in the latter case, higher ($p<0.05$) in the rats injected with a lipid emulsion (68.0±4.8 μg/g; n=12) than in the uninjected ω3-depleted rats (51.9±1.4 μg/g; n=6). Except for obvious gender differences in the C16:0, C16:1ω7, C18:0 and C18:1ω9 content of phospholipid, as well as in the C16:1ω7/C16:0 and C18:1ω9/C18:0 ratio, there was little to distinguish for the same variables between uninjected rats and animals injected with a lipid emulsion (Table I). Likewise, except for gender differences, the weight percentages of C20:0, C22:0 and C24:0 were comparable in male uninjected rats and animals injected

Table I. Fatty acid total content and pattern of liver phospholipids.

Rats	M-NI	M-OO	M-FO	F-NI	F-FO
Total fatty acid (mg/g)	18.84±0.48 (6)	19.39±0.28 (6)	20.42±1.20 (6)	22.27±0.48 (2)	19.88±1.06 (3)
C20:5ω3 (μg/g)	0.0±0.0 (6)	0.0±0.0 (6)	84.3±7.3 (6)	0.0±0.0 (2)	64.0±15.9 (3)
C22:5ω3 (μg/g)	21.0±4.5 (6)	29.0±0.9 (6)	58.6±1.7 (6)	31.9±4.4 (2)	70.3±10.4 (3)
C22:6ω3 (μg/g)	156.7±11.3 (6)	238.0±14.6 (6)	305.4±19.7 (6)	386.6±2.7 (2)	470.5±42.0 (3)
C22:5ω3/C22:6ω3 (%)	16.07±0.77 (5)	12.33±0.49 (6)	19.47±1.01 (6)	8.25±1.20 (2)	14.85±1.44 (3)
C8:0 (μg/g)	0.0±0.0 (6)	0.0±0.0 (6)	0.0±0.0 (6)	0.0±0.0 (2)	11.4±1.0 (3)
C10:0 (μg/g)	0.0±0.0 (6)	5.1±3.5 (6)	4.4±2.8 (6)	0.0±0.0 (2)	23.2±2.6 (3)
C14:0 (μg/g)	51.9±1.4 (6)	68.8±4.1 (6)	67.2±9.1 (6)	30.3±0.2 (2)	28.1±3.8 (3)
C16:0 (%)	18.9±0.2 (5)	20.2±0.4 (6)	19.0±0.5 (6)	15.2±0.9 (2)	14.0±0.4 (3)
C16:1ω7 (%)	1.3±0.1 (5)	1.3±0.1 (6)	1.2±0.2 (6)	0.5±0.1 (2)	0.4±0.0 (3)
C16:1ω7/C16:0 (x10^3)	65.9±3.9 (5)	65.5±5.7 (6)	69.2±8.3 (6)	30.9±1.0 (2)	25.4±0.9 (3)
C18:0 (%)	19.4±0.8 (6)	19.1±0.5 (6)	17.6±0.8 (6)	27.6±0.4 (2)	26.1±0.4 (3)
C18:1ω9 (%)	3.9±0.4 (6)	3.6±0.2 (6)	3.5±0.1 (6)	2.4±0.0 (2)	2.7±0.2 (3)
C18:1ω9/C18:0	0.181±0.016 (5)	0.188±0.011 (6)	0.200±0.017 (6)	0.088±0.002 (2)	0.104±0.008 (3)
C20:0 (%)	0.135±0.013 (6)	0.106±0.007 (6)	0.112±0.005 (6)	0.086±0.003 (2)	0.134±0.003 (3)
C22:0 (%)	0.336±0.013(6)	0.301±0.009 (6)	0.306±0.005 (6)	0.376±0.012 (2)	0.430±0.004 (3)
C24:0 (%)	0.786±0.017 (6)	0.741±0.025 (6)	0.760±0.021 (6)	0.861±0.019 (2)	1.070±0.047 (3)
C20:1ω9 (ppm)	903±340 (6)	1049±86 (6)	1379±156 (6)	598±42 (2)	2309±355 (3)
C22:1ω9 (ppm)	1254±706 (5)	1586±472 (6)	2588±219 (5)	429±79 (2)	741±56 (3)
C18:2ω6 (%)	12.8±0.8 (6)	11.9±0.3 (6)	14.5±0.1 (6)	9.8±0.9 (2)	11.1±0.4 (3)
C18:3ω6 (%)	0.4±0.1 (6)	0.3±0.0 (6)	0.2±0.0 (6)	0.2±0.0 (2)	0.2±0.0 (3)
C20:2ω6 (%)	0.4±0.1 (6)	0.4±0.0 (6)	0.5±0.0 (6)	0.3±0.1 (2)	0.4±0.0 (3)
C20:3ω6 (%)	1.1±0.2 (6)	0.8±0.1 (6)	0.7±0.1 (6)	0.6±0.0 (2)	0.0±0.0 (3)
C20:4ω6 (%)	37.3±1.5 (6)	38.4±0.3 (6)	37.8±0.6 (6)	38.9±0.5 (2)	38.6±0.8 (3)
C22:4ω6 (%)	0.9±0.0 (6)	1.0±0.0 (6)	0.9±0.4 (6)	1.2±0.0 (2)	1.4±0.0 (3)

either with the MCT:OO or MCT:FO emulsion. In the female rats injected with the MCT:FO emulsion, however, the weight percentage of these three long-chain saturated fatty acids was significantly higher ($p<0.05$) than in the uninjected female animals (Table I).

In male and female ω3-depleted rats, the prior injection of the MCT:FO emulsion increased the relative weight content of liver phospholipids in C20:1ω9 and C22:1ω9 respectively to 222.9±45.4% (n=9; $p<0.01$) and 177.5±10.2% (n=8; $p<0.025$) of the mean corresponding values otherwise found in the non-injected and, when available, MCT:OO-injected animals of the same sex, i.e. 100.0±14.7% (n=14) and 100.0±23.0% (n=13). In the nine rats injected with the MCT:FO emulsion, there was a significant positive correlation (r=0.685; n=9; $p<0.05$) between the weight percentage of C20:5ω3 in phospholipids and their C22:1ω9 content, both variables being expressed relative to the mean corresponding value found in animals of the same sex (Fig. 1).

Last, as far as the long-chain polyunsaturated ω6 fatty acids are concerned, the sole consistent changes provoked by the prior injection of the MCT:FO emulsion consisted in an increase of the C18:2ω6 weight percentage ($p<0.02$) to 116.2±3.0% (n=9) of the mean corresponding values found in the other animals of the same sex (100.0±4.3%; n=14), and a decrease of the C20:3ω6 weight percentage ($p<0.01$) to 50.3±13.8% (n=9) of the mean corresponding control values recorded in animals of the same gender (100.0±10.3%; n=14). The latter change was obvious, however, only in the female animals.

Liver triglycerides. The total fatty acid content of triglycerides was quite variable ranging between the extreme individual values of 7.7 and 86.3 mg/g wet weight in the male rats and 7.0 and 20.0 mg/g wet weight in the female animals. In the rats injected with the MCT:FO emulsion, it represented no more than 41.2±4.9% (n=9; $p<0.05$) of the mean corresponding values found in uninjected ω3-depleted rats of the same sex (100.0±27.1%; n=8).

In both male and female rats, the weight percentage of C16:1ω7 in the liver triglycerides increased as a function of their total fatty acid content. For instance, in the uninjected ω3-depleted rats, and those injected with the control MCT:OO emulsion, there was a positive correlation (r=0.667; n=14; $p<0.01$) between the weight percentage of C16:1ω7 and logarithm of the total fatty acid content of triglycerides, both expressed relative to the mean value found in rats of the same sex. Such a positive correlation was not ruled, however, by a relationship of proportionality between the two variables

Table II. Fatty acid total content and pattern of liver triglycerides.

Rats	M-NI	M-OO	M-FO	F-NI	F-FO
Total fatty acid (mg/g)	34.91±12.57 (6)	11.30±1.08 (6)	11.13±0.63 (6)	15.01±5.00 (2)	8.98±1.00 (3)
Log$_e$ total fatty acid					
C16:1ω7 (%)	0.35±0.03 (6)	0.34±0.06 (6)	0.47±0.10 (6)	0.71±0.11 (2)	1.32±0.11 (3)
C20:5ω3 (%)	0.0±0.0 (6)	0.0±0.0 (6)	1.7±0.1 (6)	0.0±0.0 (2)	2.9±0.9 (3)
C22:5ω3 (%)	0.0±0.0 (6)	0.0±0.0 (6)	0.7±0.1 (6)	0.0±0.0 (2)	1.0±0.3 (3)
C22:6ω3 (%)	0.0±0.0 (6)	0.0±0.0 (6)	2.0±0.2 (6)	0.1±0.1 (2)	3.0±1.0 (3)
C8:0 (%)	0.0±0.0 (6)	1.4±0.3 (6)	1.7±0.4 (6)	0.0±0.0 (2)	6.0±0.8 (3)
C10:0 (%)	0.0±0.0 (6)	2.2±0.4 (2)	2.4±0.5 (6)	0.0±0.0 (2)	6.0±1.1 (3)
C12:0 (%)	0.1±0.1 (6)	0.1±0.1 (6)	0.0±0.0 (6)	0.0±0.0 (2)	0.1±0.0 (3)
C14:0 (%)	1.7±0.1 (6)	1.8±0.2 (6)	1.5±0.2 (6)	0.9±0.1 (2)	0.7±0.1 (3)
C16:0 (%)	38.6±2.6 (6)	34.6±2.0 (6)	29.4±1.2 (6)	31.0±1.9 (2)	20.6±0.7 (3)
C16:1ω7 (%)	9.5±1.5 (6)	8.0±1.1 (6)	6.1±1.0 (6)	3.9±1.1 (2)	2.4±0.2 (3)
C16:1ω7/C16:0 (x10^3)	241±29 (6)	224±21 (6)	208±32 (6)	124±28 (2)	82±1 (3)
C18:0 (%)	1.8±0.2 (6)	1.8±0.1 (6)	1.6±0.2 (6)	2.2±0.1 (2)	2.4±0.2 (3)
C18:1ω9 (%)	29.3±1.7 (6)	28.5±0.9 (6)	24.0±0.7 (6)	32.4±1.7 (2)	22.2±1.0 (3)
C18:1ω9/C18:0	16.7±1.4 (6)	15.8±0.9 (6)	15.9±1.7 (6)	15.0±1.3 (2)	9.5±0.9 (3)
C18:2ω6 (%)	16.7±4.0 (6)	19.7±2.7 (6)	26.0±2.5 (6)	28.2±4.5 (2)	20.6±0.7 (3)
C18:3ω6 (%)	0.4±0.1 (6)	0.4±0.1 (6)	0.4±0.1 (6)	0.6±0.1 (2)	0.7±0.1 (3)
C20:2ω6 (%)	0.2±0.1 (6)	0.3±0.0 (6)	0.4±0.0 (6)	0.1±0.1 (2)	0.1±0.1 (3)
C20:4ω6 (%)	1.4±0.5 (6)	0.9±0.5 (6)	1.1±0.5 (6)	0.0±0.0 (2)	0.0±0.0 (3)
C22:4ω6 (%)	0.3±0.1 (6)	0.4±0.1 (6)	0.7±0.1 (6)	0.6±0.1 (2)	1.2±0.1 (3)
C20:2ω6/C18:2ω6 (x10^3)	15.4±2.4 (5)	13.3±1.1 (6)	14.5±1.0 (6)	4.3±0.1 (2)	4.9±0.9 (2)
C22:4ω6/C18:2ω6 (x10^3)	16.2±4.6 (4)	18.8±3.0 (6)	27.9±1.2 (6)	20.7±0.2 (2)	39.2±4.0 (3)

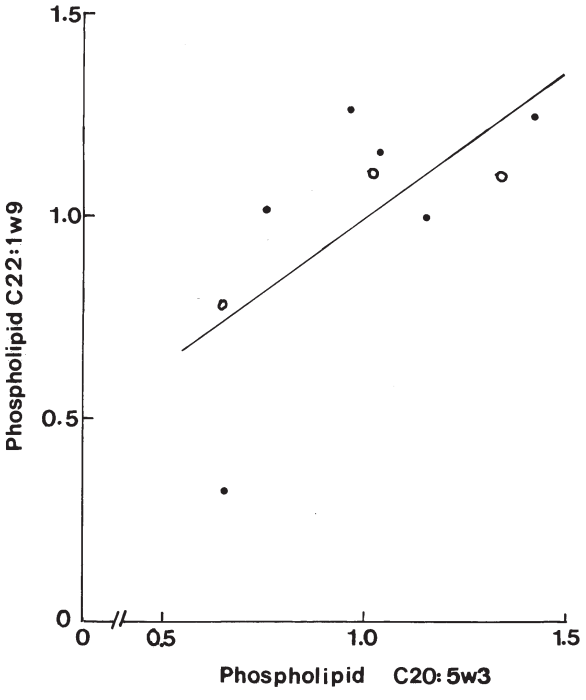

Figure 1. Correlation between the phospholipid C22:1ω9 content (μg/g) and weight percentage of C20:5ω3 in male (black circles) and female (white circles) rats injected intravenously 60 min before sacrifice with the MCT:FO emulsion. Both variables are expressed relative to the mean corresponding value found in animals of the same sex. The oblique line corresponds to the regression line.

under consideration. In the same fourteen animals, the ratio between the triglyceride fatty acid content (logarithmic value) and C16:1ω7 weight percentage was inversely related ($r=-0.671$; $n=14$; $p<0.01$) to the latter parameter, both variables being again expressed relative to the mean corresponding value found in rats of the same sex. Hence, in order to further assess the possible change in the liver triglyceride content attributable to the prior injection of the MCT:FO emulsion, the total fatty acid content of triglycerides (logarithmic value) was divided by the C16:1ω7 weight percentage measured in the same sample (Table II). According to such an analytical procedure, the results recorded in the rats injected with the MCT:FO emulsion averaged 151.4±21.5% ($n=9$; $p<0.02$) of the mean reference value found in uninjected rats (and whenever available, animals injected with the MCT:OO emulsion) of the same sex (100.0±8.2%; $n=14$). The latter finding is consistent, therefore, with the view that the prior injection of the MCT:FO emulsion 60 min before sacrifice may indeed correct the hepatic steatosis otherwise prevailing in ω3-depleted rats (2).

Except in one case, all uninjected rats and those injected with the MCT:OO emulsion were devoid of any sizeable amount of C20:5ω3, C22:5ω3 or C22:6ω3 in the liver triglycerides. Such was not the case in any of the rats injected with the MCT:FO emulsion. In these rats, the C22:5ω3/C22:6ω3 ratio was virtually identical in male (33.5±1.5%; $n=6$) and female (33.7±2.7%; $n=3$) animals.

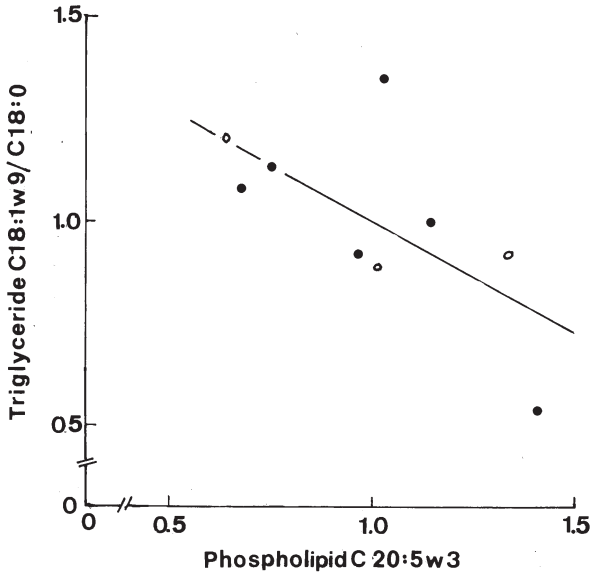

Figure 2. Correlation between the triglyceride C18:1ω9/C18:0 ratio and phospholipid C20:5ω3 weight percentage in male (black circles) and female (white circles) rats injected intravenously 60 min before sacrifice with the MCT:FO emulsion. Both variables are expressed relative to the mean corresponding value found in animals of the same sex. The oblique line corresponds to the regression line.

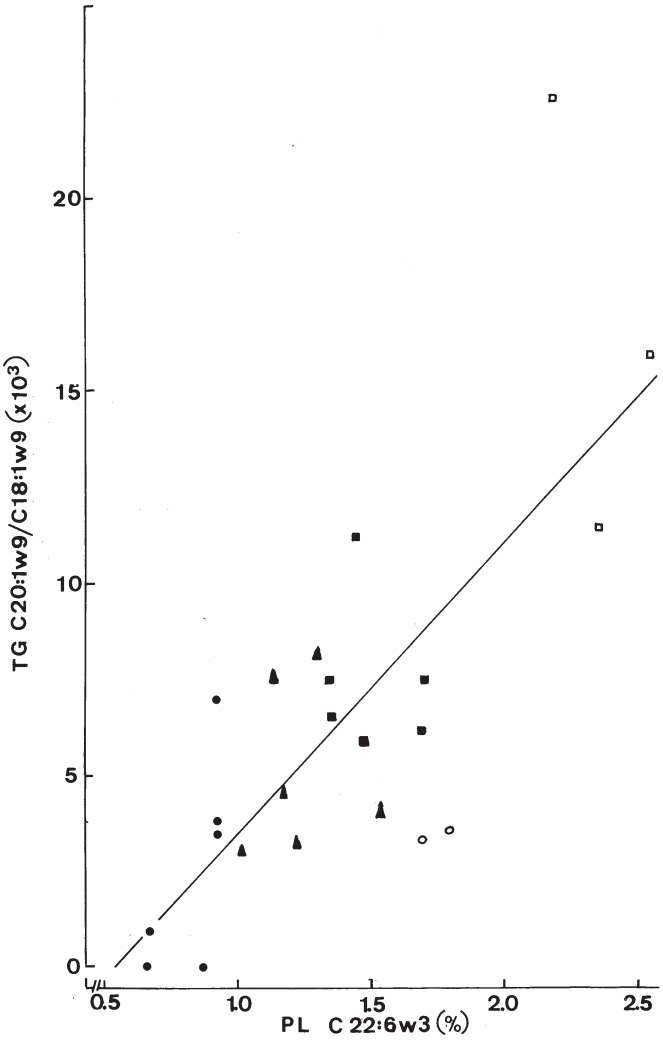

Figure 3. Relationship between the absolute values for the C22:6ω3 weight percentage of liver phospholipids (PL) and C20:1ω9/C18:1ω9 ratio in liver triglycerides of male (black symbols) or female (white symbols) ω3-depleted rats, either uninjected (circles) or injected intravenously 60 min before sacrifice with the MCT:OO (triangles) or MCT:FO (squares) emulsion.

Sizeable amounts of C8:0 and C10:0 were only found in the rats injected with a lipid emulsion (Table II). In this respect, male rats differed ($p<0.02$) from female rats, both injected with the MCT:FO emulsion, by a lower weight percentage of these middle-chain fatty acids and a lower C8:0/C10:0 ratio. Such a ratio averaged 0.646±0.037 (n=6) and 0.744±0.044 (n=6) in the male rats injected with the MCT:OO and MCT:FO emulsion, as distinct from 1.011±0.088 (n=3) in the female rats injected with the MCT:FO emulsion. It should also be underlined that, in the rats injected with a lipid emulsion, the C8:0 and C10:0 content of liver triglycerides averaged, respectively, 174.4±26.2 and 249.8±35.7 μg/g (n=12) in male rats and 534.7±99.6 and 533.3±103.8 μg/g (n=3) in female animals, as distinct from phospholipid values of only 0.0±0.0 and 4.8±2.1 μg/g (n=12) in the male rats and 11.4±1.0 and 23.2±2.6 μg/g (n=3) in the female animals. Thus, despite the fact that in these rats, the total fatty acid content of triglycerides was always lower than that of phospholipids, the enrichment of the liver lipids in C8:0 and C10:0 was, as expected, dramatically higher in triglycerides than in phospholipids.

In a mirror image of the results concerning C8:0 and C10:0, the injection of the MCT:FO emulsion lowered, in both male and female rats, the weight percentage of C14:0 in the liver triglycerides to 80.6±7.8% (n=9; $p<0.05$) of the mean corresponding value (100.0±5.1%; n=14) recorded in uninjected rats (or, when available, rats injected with the control MCT:OO emulsion) of the same sex.

As documented in Table II, the weight percentages of C16:0, C16:1ω7 and C18:1ω9 were all lower in the rats injected with the MCT:FO emulsion, than in the other animals. In the former rats, they averaged, for C16:0, C16:1ω7 and C18:1ω9 respectively, 75.7±3.2% (n=9; $p<0.001$), 67.0±7.6% (n=9; $p<0.025$) and 78.2±3.0% (n=9; $p<0.001$) of the mean corresponding values found in the other animals of the same sex (100.0±4.0%; 100.0±9.4% and 100.0±1.8%; n=14 in all cases). These three percentages found in the rats injected with the MCT:FO emulsion were not significantly different from one another ($p>0.2$), even when ignoring the dispersion of the reference measurements. The latter finding suggests that the decrease in the weight percentage of C16:0, C16:1ω7 and C18:1ω9 in the rats injected with the MCT:FO emulsion is attributable, in part at least, to the accumulation of middle-chain fatty acids (C8:0 and C10:0) and long-chain poly-unsaturated ω3 fatty acids (C20:5ω3, C22:5ω3 and C22:6ω3) in the liver triglycerides of these animals. In order to avoid the latter interference, the paired C16:1ω7/C16:0 and C18:1ω9/C18:0 ratios were calculated in the triglycerides of each animal.

Such ratios averaged in the rats injected with the MCT:FO emulsion 84.0±6.5% (n=18; $p<0.04$) of the mean corresponding

Table III. Fatty acid pattern of liver diglycerides and unesterified fatty acids.

Lipids	Diglycerides		Unesterified fatty acids	
Rats	F-NI	F-FO	F-NI	F-FO
C8:0 (%)	0.0±0.0 (2)	4.5±0.5 (3)	0.0±0.0 (2)	33.6±5.4 (3)
C10:0 (%)	0.0±0.0 (2)	6.6±0.6 (3)	0.0±0.0 (2)	11.6±1.1 (3)
C8:0/C10:0 (%)	N.D.	68.6±5.5 (3)	N.D.	286.7±25.4 (3)
C16:0 (%)	37.1±2.6 (2)	15.6±1.5 (3)	63.4±5.0 (2)	25.0±3.3 (3)
C16:1ω7 (%)	2.7±0.6 (2)	0.0±0.0 (3)	0.0±0.0 (2)	0.0±0.0 (3)
C18:0 (%)	14.9±3.5 (2)	11.8±1.0 (3)	15.0±1.9 (2)	10.2±1.2 (3)
C18:1ω9 (%)	20.3±1.2 (2)	10.1±0.7 (3)	13.4±1.8 (2)	7.4 ±1.3 (3)
C18:2ω6 (%)	22.5±2.8 (2)	14.6±1.2 (3)	5.7±5.7 (2)	10.9±3.0 (3)
C20:5ω3 (%)	0.0±0.0 (2)	10.5±2.2 (3)	0.0±0.0 (2)	0.0±0.0 (3)
C22:6ω3 (%)	0.0±0.0 (2)	13.9±1.7 (3)	0.0±0.0 (2)	0.0±0.0 (3)
C16:1ω7/C16:0 (%)	7.2±1.2 (2)	0.0±0.0 (3)	0.0±0.0 (2)	0.0±0.0 (3)
C18:1ω9/C18:0	1.5±0.4 (2)	0.9±0.1 (3)	0.9±0.2 (2)	0.7±0.1 (3)

ND, not determined.

value found in animals of the same sex either uninjected before sacrifice or injected with the control MCT:OO emulsion (100.0±3.9%; n=28). Fig. 2 illustrates the inverse correlation (r=-0.662; p=0.05) between the C20:5ω3 weight percentage in liver phospholipids and the C18:1ω9/C18:0 ratio in liver triglycerides found in the nine rats injected with the MCT:FO emulsion, both variables being expressed relative to the mean value found in animals of the same sex. Such a finding is compatible with a cause-to-effect link between the increase in the phospholipid content of long-chain polyunsaturated ω3 fatty acids, as provoked by the injection of the MCT:FO emulsion, and the restoration of a low activity of Δ9-desaturase, as otherwise prevailing in normal rats when compared to uninjected ω3-depleted animals (3). In the light of this proposal, advantage was then taken of the fact that, in liver triglycerides as distinct from liver phospholipids, both C20:5ω3 and C22:6ω3 remain below the limit of detection in the ω3-depleted rats, whether in uninjected animals or those injected with the control MCT:OO emulsion. In the female rats injected with the MCT:FO emulsion, the correlation coefficient between the triglyceride C16:1ω7/C16:0 and C18:1ω9/C18:0 ratios, respectively, and the triglyceride weight percentage of C20:5ω3 and C22:6ω3, calculated as indicated above, yielded values of -0.768 and -0.769 with a probability <0.04 for the combination of these two sets of data by covariance analysis. A comparable analysis with the male rats also injected with the MCT:FO emulsion only achieved statistical significance (r=-0.483; n=20; p<0.05) after exclusion of one animal with xy products (533±55; n=2) concerning the C16:1ω7/C16:0 ratio well above the upper limit of 95% confidence interval (i.e. the mean value plus $SD.t_{0.05}$) for individual values, as determined by the measurements made in the other five male rats (xy=-200±57; n=20).

The mean C20:0 relative content of liver triglycerides failed to differ significantly in the rats injected with the MCT:FO emulsion (1.02±0.14x10^{-3}; n=9) and in the other animals (0.73±0.10; n=14).

The C20:1ω9 relative content of liver triglycerides, however, was higher in the rats injected with the MCT:FO emulsion than in the other animals, being increased (p<0.05 in both cases) from 1.09±0.22x10^{-3} (n=12) to 1.86±0.19x10^{-3} (n=6) in male rats and from 1.12±0.10x10^{-3} (n=2) to 3.63±0.55x10^{-3} (n=3) in female animals.

Even if ignoring two null values recorded in uninjected male ω3-depleted rats, the C20:1ω9/C18:1ω9 ratio of liver triglycerides was significantly higher (p<0.05) in the male rats injected with the MCT:FO emulsion (7.29±0.69x10^{-3}; n=6) than in either the uninjected male ω3-depleted rats or those injected with the MCT:OO emulsion. The readings made in the latter two groups of rats failed to differ significantly from one another (p>0.3), and yielded an overall mean value of 3.93±0.78x10^{-3} (n=10). Likewise, the C20:1ω9/C18:1ω9 ratio of liver triglycerides was much higher (p<0.01) in female rats injected with the MCT:FO emulsion (16.03±3.17x10^{-3}; n=3) than in the uninjected ω3-depleted female animals (3.45±0.13x10^{-3}; n=2). In considering these findings, it could be objected that the same C20:1ω9/C18:1ω9 ratio was also significantly higher (p<0.01) in the plasma triglycerides of the male FO rats (14.87±2.26x10^{-3}; n=6) than in those of the male NI and OO rats (6.88±1.13x10^{-3}; n=12), so that the change of the C20:1ω9/C18:1ω9 ratio in liver triglycerides could be merely attributable to the corresponding change in plasma triglycerides. However, in the male FO rats, there was no trend towards a significant positive correlation (r=-0.0018; n=6) between the individual values for the C20:1ω9/C18:1ω9 ratio in plasma and liver. Contrasting with the latter negative finding, there was, in the male and female FO rats, a significant positive correlation (r=0.7014; n=9; p<0.04) between the absolute values for the C20:1ω9/C18:1ω9 ratio in liver triglycerides and C22:6ω3 weight percentage in liver

phospholipids. As illustrated in Fig. 3, such was also the case when all 23 rats examined in this work were considered as a whole ($r=0.7450$; $n=23$; $p<0.001$).

The prior injection of the MCT:FO emulsion failed to decrease significantly the weight percentage of any long-chain polyunsaturated ω6 fatty acid in the liver triglycerides. On the contrary, such a percentage was higher in these rats than in the other animals for C18:2ω6 ($p<0.06$), C20:2ω6 ($p<0.03$) and C22:4ω6 ($p<0.01$) in male rats. In the female animals, the C22:4ω6 weight percentage was also twice higher ($p<0.05$) in the rats injected with the MCT:FO emulsion than in the uninjected rats.

Nevertheless, as judged from the C22:4ω6/C18:2ω6 ratio, the generation of the former metabolite from its precursor was significantly higher ($p<0.05$) in both the male and female rats injected with the MCT:FO emulsion than in the other animals of the same sex. Pooling together the results obtained in male and female rats, such a ratio averaged 167.4±8.9% ($n=9$; $p<0.001$) of the mean corresponding value found in the uninjected rats and, whenever available, those injected with the control MCT:OO emulsion (100.0±11.3%; $n=12$). The latter finding contrasts with the observation that the C20:2ω6/C18:2ω6 ratio was not significantly different in uninjected rats and animals injected with either the MCT:OO or MCT:FO emulsion. There was, however, a dramatic difference ($p<0.001$) between the values for the C20:2ω6/C18:2ω6 ratio in male rats ($14.4\pm0.8\times10^{-3}$; $n=17$) and female animals ($4.6\pm0.4\times10^{-3}$; $n=4$).

In the 6 male rats and 3 female rats injected with the MCT:FO emulsion, there was a positive correlation ($p<0.06$) between the logarithmic values for the C22:4ω6/C18:2ω6 ratio and for the C20:5ω3 and C22:6ω3 weight percentages in liver triglycerides, all variables being expressed relative to the mean corresponding value found in animals of the same sex.

Liver diglycerides. The fatty acid pattern of liver diglycerides and unesterified fatty acids was also established in the female rats (Table III). The total fatty acid content of liver diglycerides averaged 367±85 μg/g ($n=5$).

The most salient differences between non-injected ω3-depleted rats and animals injected with the MCT:FO emulsion 60 min before sacrifice consisted in the presence in the latter animals, as distinct from former rats, of sizeable amounts of C8:0, C10:0, C20:5ω3 and C22:6ω3. The mean C16:1ω7/C16:0 and C18:1ω9/C18:0 ratios were lower in the F-FO rats than in the F-NI rats.

Liver unesterified fatty acids. The total fatty acid and content of liver unesterified fatty acids averaged 89.9±16.7 μg/g ($n=2$) and 156.5±19.5 μg/g ($n=3$) in the F-NI and F-FO rats, respectively. Once again, both C8:0 and C10:0 were detected in the unesterified fatty acids of F-FO rats, but not so in F-NI rats (Table III). Whilst the C8:0/C10:0 ratio was significantly lower than unity in the diglycerides of F-FO rats ($p<0.05$), a mirror image ($p<0.02$) prevailed in the unesterified fatty acids. The mean C18:1ω9/C18:0 ratio was again lower in the F-FO rats than in the F-NI rats. Taking into account the measurements made in both diglycerides and unesterified fatty acids, such a ratio averaged, in the female rats injected with the MCT:FO emulsion, 68.6±7.3% ($n=6$) of the mean corresponding values found in the uninjected ω3-depleted animals.

Discussion

In addition to confirming the severe decrease of the long-chain polyunsaturated ω3 fatty acid content of liver phospholipids and triglycerides in the second generation ω3-depleted rats (2,3), the present study affords several further major pieces of information.

First, it extends to ω3-depleted rats the knowledge of dramatic gender differences in the fatty acid pattern of liver lipids. Even the C22:6ω3 content and C22:5ω3/C22:6ω3 ratio in liver phospholipids were vastly different in the uninjected male versus female ω3-depleted rats.

Second, it unambiguously documents that, in both male and female ω3-depleted rats, the intravenous bolus injection of the MCT:FO emulsion (1.0 ml per rat) considerably augments, within 60 min, the C20:5ω3, C22:5ω3 and C22:6ω3 content of both liver phospholipids and triglycerides. This was first observed in older male animals, including both control and second generation ω3-depleted rats (2). In further work conducted mostly in relatively young ω3-depleted female rats (8-16 weeks old), the enrichment of liver lipids in long-chain polyunsaturated ω3 fatty acids was more modest. The latter situation also coincided with a lesser enrichment of liver lipids in C8:0 and C10:0 and, hence, could be related to such factors as the age of the animal, the site of injection of the lipid emulsion (with a greater risk of incomplete injection in the tail vein of conscious rats versus the splenous vein of anaesthetized rats), the length of time between injection and sacrifice, and the fact that such a time was spent either in the conscious or anesthetized state.

Last, the enrichment of liver lipids in long-chain polyunsaturated ω3 fatty acids resulting from the injection of the MCT:FO emulsion coincided with a number of other changes in the fatty acid content and/or pattern of both liver phospholipids and triglycerides. For instance, the C20:1ω9 and C22:1ω9 weight percentage of phospholipids was increased in the rats injected with the MCT:FO emulsion. As a matter of fact, in these rats, there was a significant positive correlation between the C22:1ω9 absolute content of phospholipids and their C20:5ω3 weight percentage (Fig. 1). However, the phospholipids of the MCT:FO emulsion failed to contain any detectable amount of C22:1ω9. Moreover, whenever present in detectable amounts, i.e. in 5 out of 17 cases, the weight percentage of C22:1ω9 in plasma phospholipids was not higher in FO rats (1.73±0.25‰; $n=2$) than in NI or OO rats (2.16±0.41‰; $n=3$). The C20:1ω9 relative content of liver triglycerides was also higher in the rats injected with the MCT:FO emulsion than in the other animals. This coincided, in both male and female, with a higher C20:1ω9/C18:1ω9 ratio in the liver triglycerides of FO rats, as compared to uninjected ω3-depleted or OO rats. The latter ratio, however, was also higher in the plasma triglycerides of male FO rats, as compared to NI and OO ω3-depleted male animals. These findings should be considered in the light of a possible participation of nervonic acid in the beneficial effects of ω3 fatty acids on obesity-related risk factors (4).

Likewise, the prior injection of the MCT:FO emulsion lowered the triglyceride C16:1ω7/C16:0 and C18:1ω9/C18:0 ratios, there being again in the same rats significant but negative correlations between such ratios and the C20:5ω3 weight percentage of phospholipids (Fig. 2). The statistical significance of such correlations was even higher when all ratios under consideration were first converted, as should theoretically be the case, to their logarithmic values. For instance, even when considering only the three female rats injected with the MCT:FO emulsion, such a procedure yielded coefficients of correlation between the liver triglyceride ratios relevant to the activity of Δ9-desaturase (C16:1ω7/C16:0 and C18:1ω9/C18:0 ratios) and the relative content of long-chain polyunsaturated ω3 fatty acids (C20:5ω3, C22:5ω3 and C22:6ω3) in either liver phospholipids or triglycerides amounting, respectively, to -0.5302 and -0.6860, with corresponding probabilities <0.03 and 0.003. This is compatible with the view that the administration of the MCT:FO emulsion to the ω3-depleted rats lowered the activity of Δ9-desaturase towards normal values.

The C22:4ω6/C18:2ω6 ratio in triglycerides was also increased, in both male and female rats, after injection of the MCT:FO emulsion, with, in the same animals, a positive correlation between the logarithmic values for such a ratio and for the triglyceride C20:5ω3 and C22:6ω3 weight percentage. In other words, the enrichment of liver lipids in long-chain polyunsaturated ω3 fatty acids, as resulting from the injection of the MCT:FO emulsion, apparently favoured the stepwise conversion of C18:2ω6 to C22:4ω6.

Last, the total triglyceride content in fatty acids was 2-3 times lower in the ω3-depleted rats injected with the MCT:FO emulsion than in the uninjected ω3-depleted rats. In this respect, the following remarks should be stressed. First, the total fatty acid content of liver triglycerides here recorded in the uninjected ω3-depleted rats and those injected with the control MCT:OO emulsion (22.0±6.0 mg/g; n=14) was about thrice higher ($p<0.005$) than that previously recorded (2,3) in male and female uninjected normal rats (6.1±0.4 mg/g; n=21). Second, in order to further assess the significance of changes in the total fatty acid content of triglycerides, advantage was taken of the positive correlation between the C16:1ω7 weight percentage of liver triglycerides and their total fatty acid content, as herein documented in the uninjected ω3-depleted rats and those injected with the control MCT:OO emulsion. Likewise, in a larger series of 18 uninjected ω3-depleted female rats, there was a significant positive correlation ($r=0.541$; $p<0.05$) between the C16:1ω7 weight percentage of liver triglycerides and their total fatty acid content (3).

By relating to total fatty acid content of liver triglycerides (logarithmic value) to their C16:1ω7 weight percentage, it became possible to document that such a ratio, which is inversely related to the triglyceride C16:1ω7 content, is significantly higher in ω3-depleted rats injected with the MCT:FO emulsion than in the other ω3-depleted animals. In other words, in the former animals, such a ratio moved towards the higher value otherwise found in normal animals when compared to uninjected ω3-depleted rats (3).

In conclusion, the present study reveals that the bolus intravenous injection of the MCT:FO emulsion to ω3-depleted rats not only allows, within 60 min, a sizeable increase in the long-chain polyunsaturated ω3 fatty acid content of liver lipids, but also modifies toward normalization several variables of hepatic lipids, such as their content in precursors of nervonic acid, apparent activity of Δ9-desaturase, stepwise conversion of C18:2ω6 to C22:4ω6, and total triglyceride content.

Acknowledgements

This study was supported by a grant (3.4574.07) from the Belgian Foundation for Scientific Medical Research. We are grateful to A. Chwalik and A. Dufour for technical assistance, and to C. Demesmaeker for secretarial help.

References

1. Carpentier YA, Peltier S, Portois L, Sener A and Malaisse WJ: Rapid lipid enrichment in ω3 fatty acids: Plasma data. Int J Mol Med 21: 355-365, 2008.
2. Portois L, Peltier S, Chardigny JM, Sébédio JL, Leverve X, Malaisse WJ and Carpentier YA: Rapid incorporation of long-chain polyunsaturated ω3 fatty acids and lowering of triglycerides in the liver of ω3-depleted rats injected with a medium-chain triglyceride:fish oil emulsion. Eur J Physiol 450: R11, 2005.
3. Louchami K, Zhang Y, Oguzhan B, Delporte C, Portois L, Carpentier YA, Genten F, Danguy A, Malaisse WJ and Sener A: Rapid changes in liver lipid composition and pancreatic K^+ handling and secretory behaviour provoked by the intravenous administration of a medium-chain triglyceride:fish oil emulsion to long-chain polyunsaturated ω3 fatty acid-depleted rats. Int J Mol Med 18: 1047-1055, 2006.
4. Carpentier YA, Portois L and Malaisse WJ: Reply to E. Oda. Am J Clin Nutr 85: 919-920, 2007.