Rapid changes in liver lipid composition and pancreatic islet 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

KARIM LOUCHAMI[1], YING ZHANG[1], BERRIN OGUZHAN[1], CHRISTINE DELPORTE[2], LAURENCE PORTOIS[3], YVON A. CARPENTIER[3], FRANCK GENTEN[1], ANDRE DANGUY[1], WILLY J. MALAISSE[1] and A. SENER[1]

Laboratories of [1]Experimental Hormonology, [2]Biological Chemistry and Nutrition, and [3]Surgery, Brussels Free University, B-1070 Brussels, Belgium

Received July 31, 2006; Accepted September 22, 2006

Abstract. Second generation rats depleted in long-chain polyunsaturated ω3 fatty acids are currently used as an animal model for the insufficient dietary supply of such fatty acids often prevailing in Western populations. The present study deals mainly with the effects of a novel medium-chain triglyceride: fish oil emulsion (MCT:FO), as compared to a control medium-chain triglyceride:olive oil emulsion (MCT:OO), administered as an intravenous bolus to the ω3-depleted rats 60-120 min before sacrifice upon selected biochemical and biophysical variables. The major findings consisted of a severe decrease of the ω3 fatty acid content of liver lipids in non-injected ω3-depleted rats and its partial correction after injection of the MCT:FO emulsion. The ω3-depleted rats also displayed liver steatosis, increased incorporation of long-chain polyunsaturated ω6 fatty acids in liver phospholipids and increased activity of liver Δ9-desaturase. As judged from the effects of ouabain upon ^{86}Rb net uptake by isolated pancreatic islets, the activity of Na^+,K^+-ATPase was virtually abolished in the ω3-depleted rats. The latter defect was corrected by prior intravenous injection of the MCT:FO emulsion, this coinciding with suppression of the excessive secretory response to a number of insulin secretagogues otherwise observed in the islets of ω3-depleted rats injected or not with the MCT:OO emulsion.

Correspondence to: Professor Willy 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: long-chain polyunsaturated ω3 fatty acid-depleted rats, medium-chain triglyceride:fish oil emulsion, liver phospholipid and triglyceride fatty acid content and pattern, ^{86}Rb net uptake and insulin secretion by isolated pancreatic islets

Introduction

Second generation rats depleted in long-chain polyunsaturated ω3 fatty acids (ω3 rats) are currently used as an animal model for the insufficient dietary supply of such fatty acids often prevailing in Western populations (1,2). Previous investigations have documented that these ω3 rats display several features of metabolic syndrome including visceral obesity (3), liver steatosis (4), insulin resistance (5), hypertension (6) and cardiac hypertrophy (7). Perturbation of metabolic, ionic and secretory variables were also observed in the pancreatic islets of ω3 rats (5,8,9).

A rapid correction of some of these anomalies may be achieved by the bolus intravenous injection of a novel medium-chain triglyceride:fish oil emulsion (MCT:FO). Such a procedure was indeed shown to provoke within 60 min an increase in the phospholipid content of long-chain poly-unsaturated ω3 fatty acids in platelets and leukocytes of human subjects (10) and livers of both normal and ω3-depleted rats (4). The MCT:FO emulsion was designed as a tool for the possible rapid prevention of pathological events resulting from the depletion in ω3 fatty acids, for instance cardiac arrhythmia in selected human subjects undergoing urgent anesthesia and/or surgery.

With this background information in mind, the present study was aimed mainly at investigating the effect of the bolus intravenous injection of MCT:FO to ω3 rats, 60-120 min before sacrifice, upon such variables as the liver phospholipid and triglyceride fatty acid pattern, and both the handling of K^+ and secretion of insulin by pancreatic islets isolated from the ω3-depleted rats. Control experiments included the bolus intravenous injection, under the same experimental conditions, of a medium-chain triglyceride:olive oil emulsion (MCT:OO).

Materials and methods

Fed female second generation ω3-depleted rats obtained as previously described (5) were compared to fed female normal rats of comparable mean age. The composition of the diets

Table I. Plasma D-glucose concentration in ω3-rats.

Rats		Group 1	Group 2	Group 3
ω3-NI	not injected	14.46±0.51 (16)	13.34±0.45 (16)	11.70±0.34 (12)
ω3-OO	60 min	14.66±0.73 (8)	12.74±0.45 (8)	14.15±0.70 (8)
	120 min	12.98±0.41 (4)	12.24±0.71 (4)	12.75±0.57 (4)
ω3-FO	60 min	13.43±0.99 (8)	12.72±0.60 (8)	12.58±0.48 (8)
	120 min	13.74±1.12 (4)	11.83±0.16 (4)	13.55±0.32 (4)

All results are expressed as mM. Rats examined 60 or 120 min after intravenous injection of a lipid emulsion.

Table II. Plasma insulin concentration in ω3-rats.

Rats		Group 1	Group 2	Group 3
ω3-NI	not injected	1.05±0.07 (16)	0.87±0.06 (16)	0.90±0.06 (12)
ω3-OO	60 min	1.06±0.08 (8)	0.98±0.05 (8)	0.96±0.06 (8)
	120 min	1.10±0.17 (4)	0.78±0.08 (4)	0.98±0.10 (4)
ω3-FO	60 min	1.12±0.13 (8)	1.00±0.06 (8)	0.95±0.10 (8)
	120 min	0.94±0.12 (4)	0.90±0.02 (4)	0.98±0.06 (4)

All results are expressed as ng/ml. Rats examined 60 or 120 min after intravenous injection of a lipid emulsion.

offered to the control and ω3-depleted rats was previously described (5). The ω3-depleted rats consisted of three groups of animals (groups 1, 2 and 3) age 8-9, 15-16 and 22-23 weeks, respectively. Some of the ω3-depleted rats were injected intravenously 60-120 min before sacrifice with 1.0 ml of either the MCT:FO or MCT:OO emulsion. The composition of these two emulsions was also previously reported (5).

The plasma D-glucose (11) and insulin (12) concentrations were measured by previously described methods.

For morphological studies, livers were rapidly collected and samples fixed in Bouin's fluid for 24 h. Following fixation, tissues were gradually dehydrated using a series of graded alcohols and embedded in paraffin wax. Five-micron thick histological slides were stained with either hematoxylin and eosin for general aspect or the periodic acid-Schiff's reagent for glycogen identification (13).

Liver samples were also examined for their phospholipid and triglyceride fatty acid content and pattern, using a previously reported procedure (14).

Pancreatic islets were isolated by the collagenase method (15) for measurement of their protein (16) and insulin (15) content. Groups of 10 islets each were preincubated for 15-20 min in a non-radioactive medium and then incubated for 10-60 min in the presence of $^{86}Rb^{+}$ and used to measure the net uptake of the latter tracer (17). Likewise, groups of 8 islets each were incubated for 60 min to measure insulin secretion (15).

All results are presented as mean values (± SEM) together with either the number of separate determinations (n) or degree of freedom (d.f.). The statistical significance of differences between mean values was assessed by use of Student's t-test.

Results

Body weight. At sacrifice the body weight averaged 199.9±2.4 g and 216.8±2.9 g at weeks 8 and 9 (n=20 in both cases, group 1), 261.3±3.9 g and 266.2±3.2 g at weeks 15 and 16 (n=20 in both cases, group 2) and 287.7±4.0 g (n=20; group 3) and 279.6±4.3 g (n=16; group 3) at weeks 22 and 23.

Plasma D-glucose and insulin concentration. As indicated in Table I, the plasma D-glucose concentration at sacrifice failed to differ significantly in the ω3-FO or ω3-OO rats, whether in groups 1, 2 or 3. In the ω3-FO and ω3-OO rats, the mean values recorded 60 and 120 min after injection of the lipid emulsion also failed to differ significantly from one another. The sole significant difference in plasma D-glucose concentration consisted of a decrease ($p<0.005$) of the overall mean value from 14.07±0.34 mM (n=40) in group 1 to 12.83±0.25 mM (n=40) and 12.76±0.27 mM (n=36) in groups 2 and 3, respectively.

A comparable situation prevailed in the case of the plasma insulin concentration (Table II). Indeed, the sole significant difference in plasma insulin concentration consisted of a decrease ($p<0.05$ or less) of the overall mean value from 1.06±0.05 ng/ml (n=40) in group 1 to 0.91±0.03 ng/ml (n=40) and 0.94±0.03 ng/ml (n=36) in groups 2 and 3, respectively.

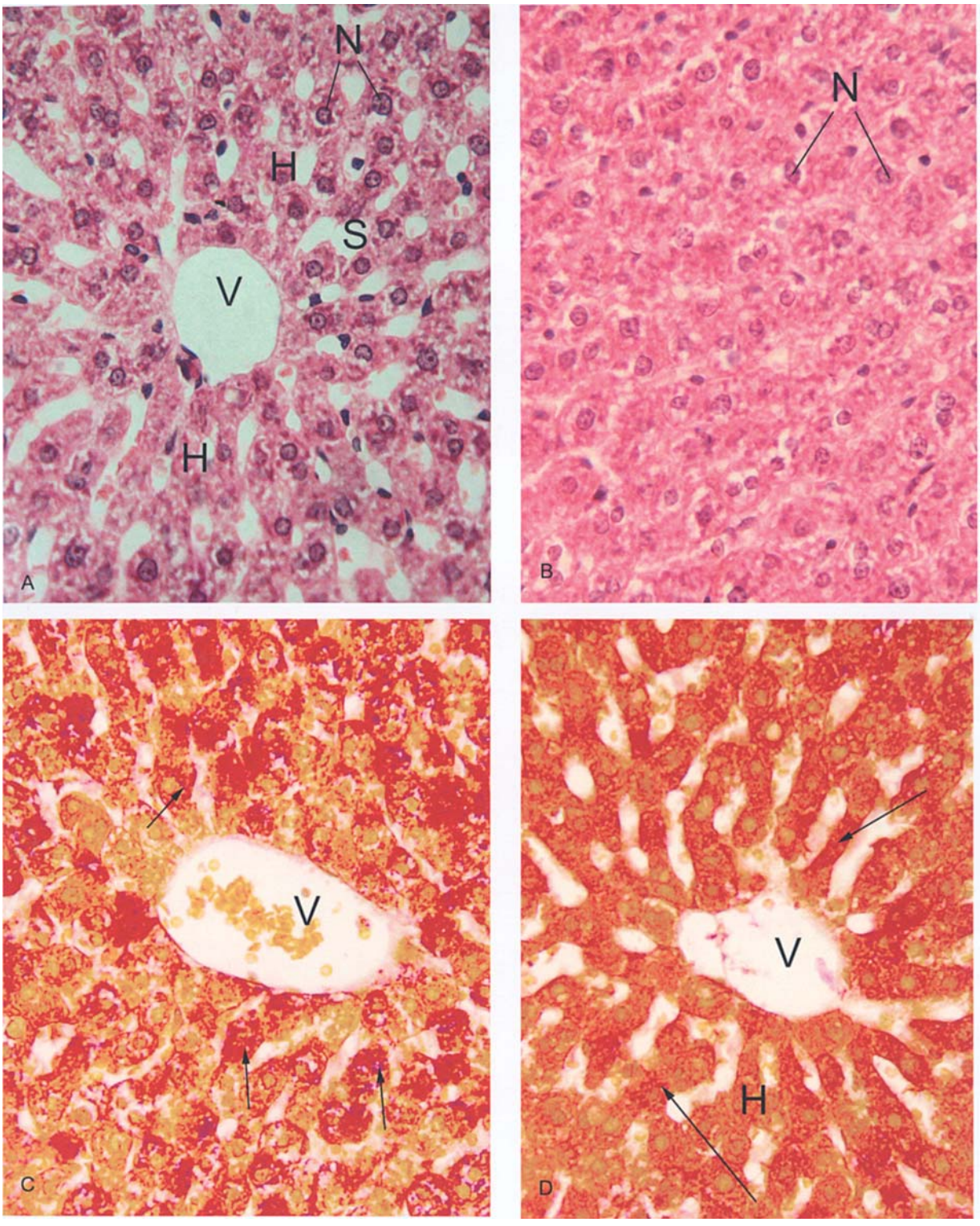

Figure 1. Micrographs stained with either hematoxylin and eosin (A and B) or periodic acid-Schiff's reagent and hematoxylin (C and D) and illustrating single liver lobules consisting of anastomosing plates of hepatocytes (H), between which are the hepatic sinusoids (S). Terminal hepatic venules (V), cell nuclei (N) and glycogen granules (arrows) are also shown. No difference was found between control rats (A and C) and ω3-depleted rats (B and D).

Liver morphology. No obvious difference was found between control and ω3-depleted rats in liver micrographs stained with either hematoxylin and eosin or periodic acid-Shiff (for glycogen identification) and hematoxylin (Fig. 1).

Liver phospholipid and triglyceride content and fatty acid pattern. In order to assess the effect of the MCT:OO and MCT:FO emulsion upon the phospholipid and triglyceride content and fatty acid pattern, these variables were measured in the livers of the ω3 rats injected with one of these emulsions. For the purpose of comparison, the results obtained in the liver of normal rats and ω3-NI rats are also presented in Table III.

The total fatty acid content of liver triglycerides was twice higher ($p<0.001$) in ω3 rats (12.2±0.4 mg/g; n=78) than in

Table III. Fatty acid content and pattern of liver lipids.

Rats	Control	ω3-NI	ω3-OO	ω3-FO
Phospholipids				
Total (mg/g)	27.06±0.64 (16)	19.28±0.24 (18)	20.53±0.34 (30)	20.12±0.37 (30)
C18:3ω3 (%)	0.16±0.01 (16)	0.00±0.00 (18)	0.00±0.00 (30)	0.00±0.00 (30)
C20:5ω3 (%)	0.52±0.04 (16)	0.00±0.00 (18)	0.00±0.00 (30)	0.06±0.03 (30)
C22:5ω3 (%)	1.44±0.06 (16)	0.14±0.01 (18)	0.15±0.01 (30)	0.19±0.02 (30)
C22:6ω3 (%)	15.16±0.45 (16)	1.60±0.06 (18)	1.62±0.06 (30)	1.77±0.09 (30)
C18:2ω6 (%)	12.68±0.30 (16)	11.29±0.28 (18)	11.13±0.18 (30)	11.21±0.22 (30)
C18:3ω6 (%)	0.14±0.01 (16)	0.31±0.01 (18)	0.31±0.01 (30)	0.31±0.01 (30)
C20:2ω6 (%)	0.29±0.02 (16)	0.31±0.01 (18)	0.30±0.01 (30)	0.32±0.01 (30)
C20:3ω6 (%)	0.63±0.03 (16)	0.87±0.04 (18)	0.74±0.04 (30)	0.79±0.04 (30)
C20:4ω6 (%)	29.52±0.34 (16)	38.31±0.26 (18)	38.69±0.23 (30)	38.22±0.23 (30)
C22:4ω6 (%)	0.31±0.01 (16)	1.21±0.03 (18)	1.23±0.02 (30)	1.21±0.03 (30)
C16:0 (%)	12.16±0.20 (16)	14.69±0.13 (18)	14.32±0.13 (30)	14.58±0.19 (30)
C16:1ω7 (%)	0.18±0.01 (16)	0.57±0.04 (18)	0.50±0.03 (30)	0.57±0.03 (30)
C18:0 (%)	23.38±0.22 (16)	25.79±0.27 (18)	26.22±0.22 (30)	25.85±0.22 (30)
C18:1ω9 (%)	1.94±0.04 (16)	2.88±0.09 (18)	2.84±0.07 (30)	3.00±0.09 (30)
Triglycerides				
Total (mg/g)	6.54±0.35 (16)	11.08±0.95 (18)	11.69±0.55 (30)	13.49±0.81 (30)
C18:3ω3 (%)	2.32±0.06 (18)	0.00±0.00 (18)	0.00±0.00 (30)	0.04±0.02 (30)
C20:5ω3 (%)	0.93±0.04 (16)	0.00±0.00 (18)	0.00±0.00 (30)	0.29±0.17 (30)
C22:5ω3 (%)	1.98±0.10 (16)	0.01±0.01 (18)	0.01±0.01 (30)	0.12±0.06 (30)
C22:6ω3 (%)	5.55±0.43 (16)	0.00±0.00 (18)	0.00±0.00 (30)	0.27±0.16 (30)
C18:2ω6 (%)	36.79±0.83 (16)	25.09±1.84 (18)	28.02±1.33 (30)	26.91±1.56 (30)
C18:3ω6 (%)	0.46±0.02 (16)	0.73±0.05 (18)	0.85±0.05 (30)	0.79±0.03 (30)
C20:2ω6 (%)	0.56±0.04 (16)	0.20±0.02 (18)	0.22±0.02 (30)	0.21±0.01 (30)
C20:3ω6 (%)	0.26±0.10 (16)	0.21±0.04 (18)	0.37±0.03 (30)	0.29±0.04 (30)
C20:4ω6 (%)	4.88±0.25 (16)	2.11±0.24 (18)	2.86±0.26 (30)	2.54±0.24 (30)
C22:4ω6 (%)	1.21±0.09 (16)	0.35±0.07 (18)	0.38±0.06 (30)	0.26±0.06 (30)
C16:0 (%)	23.21±0.87 (16)	32.47±1.09 (18)	30.10±0.82 (30)	29.97±0.88 (30)
C16:1ω7 (%)	1.09±0.11 (16)	4.62±0.47 (18)	3.95±0.24 (30)	4.46±0.28 (30)
C18:0 (%)	2.44±0.08 (16)	2.23±0.12 (18)	2.00±0.07 (30)	1.98±0.10 (30)
C18:1ω9 (%)	17.35±0.59 (16)	30.48±0.66 (18)	29.94±0.63 (30)	30.06±0.76 (30)

normal animals (6.5±0.3 mg/g; n=16). Unless otherwise mentioned, there was in this and further comparisons no significant difference between ω3-NI, ω3-OO and ω3-FO rats. The evidence of liver steatosis is reinforced by the unexpected finding of a lower total fatty acid content of liver phospholipids in ω3-NI versus control rats ($p<0.001$).

There were significant correlations between the total amount of fatty acids in liver triglycerides and their content in selected fatty acids. To cite only one example, in the ω3-NI rats, the correlation between the total fatty acid content of triglycerides and the relative contribution of C16:0, C18:2ω6 and C20:4ω6 (n=18 in all cases) amounted respectively to +0.667 ($p<0.01$), -0.551 ($p<0.02$) and -0.513 ($p<0.05$).

In the phospholipids of ω3-NI and ω3-OO rats, the C18:3ω3, C20:5ω3, C22:5ω3 and C22:6ω3 weight percentage was much lower than in control rats. No C18:3ω3 and C20:5ω3 could indeed be detected in the phospholipids of ω3-NI and ω3-OO rats, whilst the relative contributions of C22:5ω3 and C22:6ω3 were ten times lower in these ω3-rats than in control animals. Likewise, in the liver triglycerides of ω3-NI and ω3-OO rats, no long-chain polyunsaturated ω3 fatty acids were detected in sharp contrast to the situation found in control rats. Prior intravenous injection of the MCT:FO emulsion 60-120 min before sacrifice augmented, modestly but significantly, the content of liver phospholipids and triglycerides in the long-chain polyunsaturated ω3 fatty acids. For instance, in

Table IV. Ratio between distinct fatty acids in liver lipids.

Rats	Control	ω3-NI	ω3-OO	ω3-FO
Phospholipids				
C18:3ω6/C18:2ω6 (x10^3)	10.4±0.8 (16)	27.4±1.7 (18)	28.1±1.2 (30)	28.5±1.3 (30)
C20:3ω6/C18:3ω6	5.03±0.24 (16)	2.92±0.16 (18)	2.48±0.14 (30)	2.56±0.10 (30)
C20:4ω6/C20:3ω6	48.2±2.8 (16)	46.3±2.6 (18)	56.3±2.9 (30)	50.9±2.3 (30)
C22:4ω6/C20:4ω6 (x10^3)	10.3±0.3 (16)	31.7±0.6 (18)	31.7±0.6 (30)	31.6±0.8 (30)
C16:1ω7/C16:0 (x10^3)	15.5±0.8 (16)	39.0±2.8 (18)	34.8±1.7 (30)	38.8±1.7 (30)
C18:1ω9/C18:0 (x10^3)	83.5±2.4 (16)	112.1±4.0 (18)	107.4±2.6 (30)	116.4±3.7 (30)
(C18:0 + C18:1ω9)/(C16:0 + C16:1ω7)	2.06±0.04 (16)	1.88±0.03 (18)	1.97±0.03 (30)	1.92±0.03 (30)
Triglycerides				
C18:3ω6/C18:2ω6 (x10^3)	13.0±0.7 (16)	31.5±2.5 (18)	31.3±1.5 (30)	31.1±1.6 (30)
C20:3ω6/C18:3ω6	1.64±0.15 (5)	0.38±0.07 (14)	0.45±0.04 (30)	0.43±0.04 (27)
C20:4ω6/C20:3ω6	5.76±0.91 (5)	7.90±0.63 (14)	8.37±0.80 (30)	8.32±0.53 (27)
C22:4ω6/C20:4ω6 (x10^3)	255.5±13.1 (16)	189.8±7.2 (15)	187.5±6.7 (22)	197.7±14.9 (15)
C16:1ω7/C16:0 (x10^3)	45.5±3.0 (16)	138.8±10.6 (18)	128.7±5.3 (30)	145.8±6.3 (30)
C18:1ω9/C18:0	7.19±0.30 (16)	14.09±0.54 (18)	15.28±0.46 (30)	15.89±0.60 (30)
(C18:0 + C18:1ω9)/(C16:0 + C16:1ω7)	0.82±0.01 (16)	0.89±0.02 (18)	0.95±0.02 (30)	0.95±0.02 (30)

the phospholipids, the weight percentage of these fatty acids was 0.25±0.08% higher ($p<0.005$) in ω3-FO than ω3-OO rats. Likewise, significant amounts of C18:3ω3, C20:5ω3, C22:5ω3 and C22:6ω3 were often detected in the triglycerides of ω3-FO rats, whilst such was never the case in ω3-OO rats. When present in detectable amounts in the triglycerides of ω3-FO rats, the absolute amount of C18:3ω3 averaged 23.8±4.0 μg/g (n=7); that of C20:5ω3, 176.9±77.4 μg/g (n=5); that of C22:5ω3, 56.0±17.8 μg/g (n=7); and that of C22:6ω3, 204.8±81.8 μg/g (n=4). The C22:6ω3/C22:5ω3 ratio of liver phospholipids was not significantly different in control rats (10.7±0.5; n=16), ω3-NI rats (12.3±0.9; n=18), ω3-OO rats (10.9±0.7; n=28) and ω3-FO rats (11.1±0.8; n=28). Likewise, whenever calculable, the C22:6ω3/C22:5ω3 ratio of liver triglycerides was not significantly different in control rats (2.57±0.10; n=16) and ω3-FO rats (2.31±0.62; n=4).

The weight percentage of long-chain polyunsaturated ω6 fatty acids was also vastly different in control and ω3 rats. Except in the case of C18:2ω6 and C20:2ω6, these fatty acids (C18:3ω6, C20:3ω6, C20:4ω6, C22:4ω6) indeed displayed a higher contribution ($p<0.001$) in the phospholipids of ω3-NI rats versus control animals. This coincided with a significant increase ($p<0.001$) of both the C18:3ω6/C18:2ω6 and C22:4ω6/C20:4ω6 ratios in liver phospholipids (Table IV). The increase in the C18:3ω6/C18:2ω6 ratio could suggest increased activity of Δ6-desaturase. As judged from the C20:4ω6/C20:3ω6 ratio, however, the activity of Δ5-desaturase would appear not to be significantly different in the ω3-NI rats and control animals. A trend towards an increase in the latter ratio ($p<0.06$) was only observed when comparing ω3 rats injected with a lipid emulsion (53.60±1.85; n=60) to ω3-NI rats (46.26±2.62; n=18). In elongase-catalyzed reactions, the product/precursor ratio was either decreased ($p<0.001$) as judged from the C20:3ω6/C18:3ω6 ratio or increased ($p<0.001$) as judged from the C22:4ω6/C20:4ω6 ratio, when comparing ω3-NI to control animals. In terms of the comparison between control and ω3-depleted rats, the C18:3ω6/C18:2ω6, C20:3ω6/C18:3ω6 and C20:4ω6/C20:3ω6 ratios displayed a comparable pattern in liver triglycerides as in liver phospholipids. Such was not the case, however, for the C22:4ω6/C20:4ω6 ratio. Because of the low to undetectable amount of C20:3ω6 in liver triglycerides, the C20:4ω6/C18:3ω6 was also computed in the latter lipids. It was much lower ($p<0.001$) in ω3-NI rats (2.83±0.30; n=18), ω3-OO rats (3.27±0.22; n=30) and ω3-FO (3.28±0.29; n=30) than in control animals (10.94±0.85; n=16).

The mean value for the weight percentage of the six long-chain polyunsaturated ω6 fatty acids listed in Table III was always lower in the triglycerides of ω3-FO as compared to ω3-OO rats. Pooling all available data, it averaged in the former rats 86.6±3.8% (n=180; $p<0.02$) of the mean corresponding value found in the latter animals (100.0±3.8%; n=180).

Last, there were also marked differences ($p<0.001$) in the weight percentage of C16:0, C16:1ω7 and C18:1ω9 in the liver phospholipids and triglycerides of ω3-NI versus control rats (Table III). In this respect, the most obvious finding consisted of the marked increase in Δ9-desaturase activity in ω3-depleted rats as judged from either the C16:1ω7/C16:0 or C18:1ω9/C18:0 ratio in both phospholipids and triglycerides (Table IV). As judged from the (C18:0 + C18:1ω9)/(C16:0 + C16:1ω7) ratio, however, the activity of elongase appeared either increased ($p<0.01$) or decreased ($p<0.005$) in the case of triglycerides and phospholipids, respectively (Table IV).

For the sake of simplicity, the results listed in Tables III and IV for the ω3-NI, ω3-OO and ω-FO rats refer to the pooled

Table V. ^{86}Rb net uptake by islets.

Rats	ω3-OO		ω3-FO	
Ouabain	Nil	50 μM	Nil	50 μM
Absolute values for ^{86}Rb net uptake (pmol/islet)				
Min 10	104.6±4.1 (48)	92.4±3.2 (48)	88.1±3.0 (47)	68.9±3.1 (43)
Min 60	204.8±7.3 (48)	153.4±5.4 (48)	211.1±10.3 (48)	155.7±7.0 (50)
Relative values in the absence/presence of ouabain (%)				
Min 10	100.0±3.4 (48)	88.4±2.5 (48)	100.0±2.9 (47)	77.6±3.0 (43)
Min 60	100.0±3.6 (48)	74.9±2.6 (48)	100.0±4.9 (48)	73.8±3.3 (50)
Relative values at min 10/min 60 (%)	52.1±2.1 (48)	61.2±2.2 (48)	44.3±2.3 (47)	47.5±2.5 (43)
Calculated values at isotopic equilibrium				
Net uptake (pmol/islet)	207.6±7.4 (48)	153.9±5.4 (48)	218.7±10.7 (48)	159.6±7.2 (50)
Fractional outflow rate (% per min)	7.2±0.3	9.4±0.5	5.6±0.3	6.2±0.4
Inflow-outflow rate (pmol/islet per min)	14.9±0.5 (48)	14.5±0.5 (48)	12.2±0.6 (48)	9.9±0.4 (50)

data collected, in each case, in groups of animals of increasing age (8-9, 15-16 and 22-23 weeks old). It should not be ignored, however, that certain variables under consideration displayed significant age-related changes. To cite only one example, the weight percentage of C22:6ω3 in the liver phospholipids of ω3-NI and ω3-OO rats, progressively increased from 1.24±0.03% in the younger rats to 1.67±0.04% in the middle-aged animals and 1.93±0.04% in the older rats (n=16 in all cases). These three values were indeed significantly different ($p<0.001$) from one another.

Islet protein and insulin content. In group 1, the islet protein content was not significantly different in ω3-OO rats versus either ω3-FO or ω3-NI rats, with an overall mean value of 1.27±0.07 μg/islet (n=19). Likewise in group 3, the islet protein content was not significantly different ($p>0.3$ or more) in the ω3-OO, ω3-NI and ω3-FO rats, with an overall mean value of 1.83±0.12 μg/islet (n=18). The latter value was higher ($p<0.001$), however, than that found in the younger rats of group 1.

In groups 1, 2 and 3, the insulin content of the islets averaged in the ω3-FO and ω3-NI rats, respectively, 98.1±7.3% (n=17) and 95.1±6.9% (n=23) of the mean corresponding value found within the same series of experiments in ω3-OO rats (100.0±9.3%; n=18). The overall mean value for the islet insulin content in group 1 (897±40 μU/islet; n=20) was higher ($p<0.001$) than that found in either group 2 (451±52 μU/islet; n=19) or group 3 (560±25 μU/islet; n=19), which failed to differ significantly from one another.

^{86}Rb net uptake. Two major differences were observed between the islets from ω3-OO and ω3-FO rats in terms of ^{86}Rb handling (Table V).

First, after only 10-min incubation, i.e. when the net uptake of ^{86}Rb informs mainly on the rate of K^+ entry into the islet cells, the values for such an uptake found in the presence of ouabain, expressed relative to the mean corresponding value recorded within the same experiment(s) in the absence of the cardiac glycoside, averaged in the ω3-OO rats 88.4±2.5% (n=48), as distinct ($p<0.01$) from only 77.6±3.1% (n=43) in the ω3-FO rats. In both cases, the ouabain-induced decrease in ^{86}Rb net uptake achieved statistical significance, amounting to 11.6±4.2% (d.f.=94; $p<0.01$) in ω3-OO rats and 22.4±4.2% (d.f.=88; $p<0.001$) in ω3-FO rats. These findings suggest that the activity of the Na,K-ATPase, as judged from the ouabain-induced decrease in ^{86}Rb net uptake, was approximately twice higher in ω3-FO rats than in ω3-OO rats.

Second, when the ^{86}Rb net uptake found after 10-min incubation was expressed relative to the mean corresponding value found within the same experiment(s) after 60-min incubation, such a ratio averaged, in the absence of ouabain, 52.1±2.1% (n=48) in ω3-OO rats, as compared ($p<0.02$) to 44.3±2.3% (n=47) in ω3-FO rats (Table I). In the presence of ouabain, the mean value for such a ratio was also higher ($p<0.001$) in ω3-OO rats (61.2±2.2%; n=48) than in ω3-FO rats (47.5±2.5%; n=43). Moreover, the cardiac glycoside augmented significantly ($p<0.005$) such a ratio in ω3-OO rats, whilst failing to do so ($p>0.3$) in ω3-FO rats. These findings indicate that the fractional turnover rate of the islet K^+ pool is lower in ω3-FO rats than in ω3-OO rats, being further increased by ouabain in the latter animals. Indeed, as derived from the equation $U = Umax (1 - e^{kt})$, in which U and Umax refer to the net uptake of ^{86}Rb at time t and at isotopic equilibrium, respectively, and K to the fractional outflow rate (18), the latter variable expressed per min^{-1} was close to 0.072 and 0.056 in the islets prepared from ω3-OO and ω3-FO rats, respectively, and incubated in the absence of ouabain, and 0.094 and 0.062 in the islets from the same animals incubated in the presence of the cardiac glycoside.

Despite such differences in fractional outflow rate, the estimated values for Umax, as also derived from the mean absolute value for ^{86}Rb net uptake at min 60 remained not significantly different in ω3-OO and ω3-FO rats, whether in the absence or presence of ouabain, with overall mean values

Table VI. Insulin release by islets from ω3-OO and ω3-FO rats incubated in the presence of 8.3 mM D-glucose.

Insulin output (unit)	Absolute values (μU/islet per 60 min)		Relative values (% of control)		Experimental/control (% of ω3-OO)	
Rats	ω3-OO	ω3-FO	ω3-OO	ω3-FO	ω3-OO	ω3-FO
Control (D-glucose 8.3 mM)	44.6±2.4 (71)	62.3±3.1 (64)[e]	100.0±4.8 (71)	100.0±2.8 (64)	-	-
Carbamylcholine (0.1 mM)	48.9±1.6 (71)	53.5±2.3 (62)	114.1±4.3 (71)	88.0±3.0 (62)[e]	100.0±2.9 (71)	77.1±2.3 (62)[e]
Ouabain (1.0 mM)	38.5±2.2 (53)	41.6±2.7 (44)	83.5±4.8 (53)	64.0±3.3 (44)[d]	100.0±4.8 (53)	79.9±3.5 (44)[d]
TPA (1.0 μM)	110.8±5.6 (74)	129.0±6.2 (62)[a]	247.7±9.7 (74)	215.6±10.1 (62)[b]	100.0±3.4 (74)	83.8±2.9 (62)[e]
Glibenclamide (5.0 μM)	65.0±3.9 (33)	65.3±4.7 (34)	130.2±6.7 (33)	106.5±5.5 (34)[c]	100.0±3.5 (33)	80.9±2.8 (34)[e]
Theophylline (1.4 mM)	174.7±5.9 (33)	178.7±7.6 (30)	372.8±25.0 (33)	315.6±25.3 (30)	100.0±2.7 (33)	81.8±2.6 (30)[e]
Cytochalasin B (20.8 μM)	119.6±7.3 (31)	125.4±6.2 (34)	262.7±23.4 (31)	216.4±15.4 (34)	100.0±5.6 (31)	85.1±3.8 (34)[a]

[a]$p<0.05$, [b]$p<0.025$, [c]$p<0.01$, [d]$p<0.005$ and [e]$p<0.001$ for the difference between ω3-OO and ω3-FO rats.

of 213.1±6.5 pmol/islet (n=96) and 156.8±4.5 pmol/islet (n=98) in the absence and presence of the cardiac glycoside, respectively.

Nevertheless, when making allowance for the difference in ^{86}Rb fractional outflow rate, the estimated inflow-outflow rate at isotopic equilibrium remained significantly lower ($p<0.005$ or less) in ω3-FO rats than ω3-OO rats, whether in the absence of ouabain (12.2±0.6 versus 14.9±0.5 pmol/islet per min) or in its presence (9.9±0.4 versus 14.5±0.5 pmol/islet per min). Most importantly, these data document that ouabain significantly decreased ($p<0.005$) $^{86}Rb^+$ inflow in the islets from ω3-FO rats, whilst failing to do so ($p>0.5$) in the islets from ω3-OO rats.

The present measurements of ^{86}Rb net uptake thus confirm that the activity of the Na^+,K^+-ATPase mediating part of ^{86}Rb influx into the islet cells is significantly higher in ω3-FO rats than in ω3-OO rats (9). Despite such a situation, the net uptake of ^{86}Rb after 10-min incubation was somewhat lower in ω3-FO than ω3-OO rats, averaging in the former animals 85.3±4.2% (n=47; $p<0.01$) of the mean corresponding values found, at the same age, in ω3-OO rats (100.0±3.4%; n=48). This apparently implies that the prior administration of the MCT:FO emulsion decreased the influx of ^{86}Rb by an ouabain-resistant modality. After 10-min incubation, the net uptake of $^{86}Rb^+$ was not only lower in absolute terms in ω3-FO than ω3-OO rats. Even when expressed relative to the corresponding value found within the same experiment(s) after 60-min incubation, it remained significantly lower in ω3-FO than ω3-OO rats. This finding is again consistent with the postulated inhibition after intravenous injection of the MCT:FO emulsion of the ouabain-resistant modality of ^{86}Rb inflow into islet cells. In turn, the lower fractional turnover rate of $^{86}Rb^+$ found in ω3-FO versus ω3-OO rats is reminiscent of the modest improvement of D-glucose metabolism found in the islets of the former, as distinct from latter animals (5). In islets from normal rats, the fractional outflow rate of $^{86}Rb^+$ is indeed decreased at increasing concentrations of extracellular D-glucose (19). Last, when the true inflow rate of ^{86}Rb was computed from the fractional turnover rate and steady-state value for ^{86}Rb net uptake at isotopic equilibrium, it became evident that ouabain decreases by approximately 20% the inflow of ^{86}Rb into the islet cells of ω3-FO rats, whilst failing to affect such an inflow in the islets of ω3-OO rats.

Secretory data. The release of insulin evoked by 8.3 mM D-glucose over 60-min incubation was significantly higher in the islets from ω3-FO rats than ω3-OO rats (Table VI). When compared within each set of experiments conducted in animals of the same age, the output of insulin averaged, in the ω3-FO rats, 138.6±4.7% (n=64; $p<0.001$) of that recorded in the ω3-OO rats (100.0±4.8%; n=71).

When the release of insulin recorded in the concomitant presence of D-glucose (8.3 mM) and another secretagogue was expressed relative to the control value measured within the same experiment in the sole presence of the hexose, all agents listed in Table VI were found to augment significantly ($p<0.01$ or less) glucose-induced insulin secretion in the islets of ω3-OO rats, except ouabain which decreased ($p<0.02$) the secretory response to the hexose. In the ω3-FO rats, however, glibenclamide failed to augment significantly insulin secretion evoked by D-glucose and carbamylcholine even impaired ($p<0.005$) the insulinotropic action of the sugar.

As judged from the ratio between the results obtained in each experiment in the presence versus absence of given secretagogues, the mean relative magnitude of the secretory response was always higher in the ω3-OO rats than in the ω3-FO rats (Table VI; relative values expressed in % of control). Moreover, when the results so obtained in the ω3-OO and ω3-FO rats were compared within each set of experiments conducted in animals of the same age (Table VI; right columns), the latter difference achieved statistical significance ($p<0.05$ or less) with all tested secretagogues. In such a respect, the least marked difference between ω3-OO and ω3-FO rats, both in quantitative and statistical terms, referred to the islets exposed to cytochalasin B.

Discussion

The present study extends prior observations also conducted in second generation ω3-depleted rats and dealing with such

items as plasma D-glucose and insulin concentration (5), liver phospholipid and triglyceride fatty acid content and pattern (4), pancreatic islet protein and insulin content (5), and both ^{86}Rb net uptake (9) and insulin secretion (5,8) by isolated islets. In these respects, emphasis is here placed on the comparison between ω3-depleted rats injected 60-120 min before sacrifice with either MCT:FO or MCT:OO.

The major findings can be described as follows. First, the present study documents an age-related decrease of both plasma D-glucose and insulin concentrations in the ω3-depleted rats, this coinciding with a progressive increase in the weight percentage of C22:6ω3 in the liver phospholipids. These results are compatible with the modulation of insulin resistance, previously documented in ω3-depleted rats (5), by the relative content of long-chain polyunsaturated ω3 fatty acids in phospholipids. In this respect, it should be underlined that the insulin content of isolated islets was also lower in older ω3-depleted rats, despite an age-related increase of the islet protein content.

Second, the measurements of liver phospholipid and triglyceride total fatty acid content confirm the presence of hepatic steatosis in the ω3-depleted rats, this coinciding with an unexpected decrease in liver phospholipid total fatty acid content. In addition to documenting the depletion of ω3 fatty acids in the liver phospholipids and triglycerides of ω3-NI rats and the partial correction of such a defect in ω3-FO rats, the fatty acid pattern of liver in ω3-NI rats was characterized by higher contributions of C18:3ω6, C20:3ω6, C20:4ω6 and C22:4ω6 than those recorded in control animals. The generation of C18:3ω6 from C18:2ω6 and that of C22:4ω6 from C20:4ω6 also appeared more efficient in ω3-NI rats than in control animals, as judged from the phospholipid ratio between relevant fatty acids.

Third, the activity of Δ9-desaturase was apparently also increased in ω3-NI rats (or other groups of ω3-depleted animals), as judged from the liver phospholipid or triglyceride C16:0/C16:1ω7 and C18:0/C18:1ω9 ratios.

Nevertheless, no obvious alteration of the general aspect or glycogen content of the liver was observed at the histological level.

The present results also confirm that ouabain significantly decreases $^{86}Rb^+$ inflow in the islets from ω3-FO rats, whilst failing to do so in the islets from ω3-OO rats. The latter finding is consistent with prior observations made in the islets of ω3-NI or ω3-OO rats and indicating a low activity of Na^+,K^+-ATPase in such islets, as judged by the effect of ouabain upon $^{86}Rb^+$ net uptake (9). In both this prior and the present study, the intravenous injection of the MCT:FO emulsion restored a sizeable activity of the latter enzyme.

Last, the secretory data collected in the present study (Table VI) again extend prior observations (5,8) indicating that, relative to the release of insulin recorded in the sole presence of 8.3 mM D-glucose, that found in the concomitant presence of other insulin secretagogues is significantly lower in the islets from ω3-FO rats than in those from ω3-OO rats. A comparable situation is found when comparing islets from control rats to those obtained from ω3-NI rats (5,8).

In conclusion, therefore, the present study reinforces the knowledge that second generation ω3-depleted rats, with low content of long-chain polyunsaturated ω3 fatty acids in tissue lipids, display liver steatosis, facilitated generation and incorporation into phospholipids of long-chain polyunsaturated ω6 fatty acids, increased activity of Δ9-desaturase, impaired activity of ouabain-sensitive Na^+,K^+-ATPase in pancreatic islets and increased responsiveness to a number of insulin secretagogues in isolated islets exposed to a physiological concentration of D-glucose. Moreover, the present results confirm that the latter two anomalies are corrected within 60-120 min by the prior injection of the long-chain poly-unsaturated ω3 fatty acid-rich MCT:FO emulsion.

Acknowledgements

This study was supported in part by a grant from the Nutricia Research Foundation (The Hague, The Netherlands), and a grant from the Région de Bruxelles-Capitale (Convention BC-BR 246/2410) to Target Hit s.a. (Brussels, Belgium). The lipid emulsions were provided by B. Braun Melsungen (Melsungen, Germany). We are grateful to J.-M. Chardigny (Laboratoire de Nutrition Humaine, Université d'Auvergne, Clermont-Ferrand, France) for the supply of ω3-depleted rats, to A. Chwalick, A. Dufour and E. Hupkens for technical assistance, and C. Demesmaeker for secretarial help.

References

1. Simopoulos A: The importance of the ratio omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56: 365-379, 2002.
2. Benatti P, Peluso G, Nicolai R and Calvani M: Polyunsaturated fatty acids: biochemical, nutritional and epigenetic properties. J Am Coll Nutr 23: 281-302, 2004.
3. Oguzhan B, Sancho V, Acitores A, Villanueva-Peñacarrillo ML, Portois L, Chardigny J-M, Sener A, Carpentier YA and Malaisse WJ: Alteration of adipocyte metabolism in ω3 fatty acid-depleted rats. Horm Metab Res (in press).
4. Portois L, Peltier S, Chardigny J, Sébédio J, Leverve X, Malaisse WJ and Carpentier YA: Correction of excessive triglyceride accumulation in the liver of ω3 fatty acid-depleted rats by single i.v. injection of a medium-chain triglyceride:fish oil emulsion. Clin Nutr 24: 565, 2005.
5. Oguzhan B, Zhang Y, Louchami K, Courtois P, Portois L, Chardigny J-M, Malaisse WJ, Carpentier YA and Sener A: Pancreatic islet function in ω3 fatty acid-depleted rats. Glucose metabolism and nutrient-stimulated insulin release. Endocrine 29: 467-476, 2006.
6. Armitage JA, Pearce AD, Sinclair AJ, Vingrys AJ, Weisinger RS and Weisinger HS: Increased blood pressure later in life may be associated with perinatal n-3 fatty acid deficiency. Lipids 38: 459-464, 2003.
7. Peltier S, Malaisse WJ, Portois L, Demaison L, Novel-Chaté V, Chardigny J-M, Sébédio JL, Carpentier YA and Leverve XM: Acute *in vivo* administration of a fish oil-containing emulsion improves post-ischemic cardiac function in ω3-depleted rats. Int J Mol Med 18: 741-749, 2006.
8. Zhang Y, Oguzhan B, Louchami K, Chardigny J-M, Portois L, Carpentier YA, Malaisse WJ, Herchuelz A and Sener A: Pancreatic islet function in ω3 fatty acid-depleted rats. Alteration of calcium fluxes and calcium-dependent insulin release. Am J Physiol (in press).
9. Malaisse WJ, Louchami K, Zhang Y, Oguzhan B, Courtois P, Portois L, Chardigny J-M, Sener A and Carpentier YA: Perturbation of K^+ fluxes in pancreatic islets from ω3-fatty-acid-depleted rats. Diabetes 54 (suppl. 1): A653, 2005.
10. Portois L, Deckelbaum RJ, Malaisse WJ and Carpentier YA: Accumulation rapide d'eicosapentaenoate dans les phospholipides cellulaires après injection intraveineuse d'une émulsion d'huile de poisson et de triglycérides à chaîne moyenne à des sujets normaux. Nutr Clin Metabol 18 (suppl. 1): S53, 2004.
11. Bergmeyer HU and Berndt E: Determination with glucose oxidase and peroxidase. In: Methods of Enzymatic Analysis. Bergmeyer HU (ed). Academic Press, New York, pp1205-1215, 1974.

12. Leclercq-Meyer V, Marchand J, Woussen-Colle MC, Giroix M-H and Malaisse WJ: Multiple effects of leucine on glucagon, insulin and somatostatin secretion from the perfused rat pancreas. Endocrinology 116: 1168-1174, 1985.
13. Hayat MA: Stains and cytochemical methods. Plenum Press, New York, p62, 1993.
14. Richelle M, Carpentier YA and Deckelbaum RJ: Long- and medium-chain triacylglycerols in neutral lipid-exchange processes with human plasma low-density lipoproteins. Biochemistry 33: 4872-4878, 1984.
15. Malaisse-Lagae F and Malaisse WJ: Insulin release by pancreatic islets. In: Methods in Diabetes Research. Larner S and Pohl SL (eds). Vol. I, part B. John Wiley & Sons, New York, pp147-152, 1984.
16. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 153: 265-275, 1951.
17. Malaisse WJ, Zhang Y, Cancelas J, Prieto PG, Olivares E, Villanueva-Peñacarrillo ML and Valverde I: Stimulation of proinsulin biosynthesis by K^+ in rat pancreatic islets. Endocrine 23: 51-57, 2004.
18. Malaisse WJ: Assessment of inflow rate, fractional outflow rate and steady-state cellular pool of ions based on two measurements of radioactive tracer net uptake. WSEAS Transactions Biol Med 3: 317-320, 2006.
19. Malaisse WJ, Boschero AC, Kawazu S and Hutton JC: The stimulus-secretion coupling of glucose-induced insulin release. XXVII. Effect of glucose on K+ fluxes in isolated islets. Eur J Physiol 373: 237-242, 1978.