Hemostatic safety of the bolus intravenous injection of a novel medium-chain triglyceride:fish oil emulsion

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Abstract. The bolus intravenous injection of a novel 8:2 medium-chain triglyceride:fish oil emulsion was recently found to increase within 60 min and for the subsequent 24-48 h the long-chain polyunsaturated ω3 fatty acid content of both leucocyte and platelet phospholipids in 12 normal subjects. The aim of the present report is to document the hemostatic safety of such a procedure in the same 12 subjects. No adverse effect was found when comparing the results obtained after administration of either the fish oil-containing emulsion or a control 5:5 medium-chain triglyceride:soybean triglyceride emulsion, whether in terms of the occlusion time in either an ADP or epinephrine test or in terms of the [CD]42b, [CD]62p, fibrinogen and PAC-1 response to ADP, collagen or thrombin receptor analog peptide 6 in platelets examined by fluorescence activated cell sorting. In conclusion, this novel procedure for the rapid enrichment of cell phospholipid in long-chain polyunsaturated ω3 fatty acids presents the required safety in a hemostatic perspective.

Introduction

The evolution of dietary lipid intake in Western populations is characterized by decreasing intakes of long-chain polyunsaturated ω3 fatty acids (1). The American Heart Association has recently proposed to increase the consumption of long-chain polyunsaturated ω3 fatty acids (2). The efficacy of dietary supplementation, however, requires a matter of days, because of the slow tissue delivery of ω3 fatty acids when orally ingested (3,4). The intravenous injection of a suitable emulsion rich in fish oil was recently proposed as a tool to increase rapidly the cell phospholipid content in ω3 fatty acids and, hence, as a procedure to protect selected patients against the risk of undesirable events, e.g. cardiac arrhythmia following anaesthesia and surgery (5). In such a perspective, a novel 20% (w/v) emulsion containing 80% medium-chain triacylglycerols and 20% fish oil was designed, taking into account the fact that medium-chain triglycerides are excellent substrates for lipoprotein lipase-mediated hydrolysis, which promptly releases medium-chain fatty acids from emulsion particles. Medium-chain triglycerides are also efficiently transferred to endogenous lipoproteins. Both processes lead to the rapid formation of small sized remnants which are promptly taken up by different tissues (6). In normal subjects, the bolus intravenous injection of 50 ml of this novel emulsion indeed increases within 60 min and for the subsequent 24-48 h the ω3 fatty acid content of both blood leucocyte and platelet phospholipids (7). The major aim of the present report is to document the hemostatic safety of such a procedure, as assessed in the same 12 normal subjects as those in whom the enrichment of circulating leucocyte and platelet phospholipids in ω3 acids was observed. The selection of this theme was motivated by the following considerations. Comparison between Inuits and Danes and between inhabitants of Japanese fishing and farming villages first showed that populations consuming high amounts of ω3 acids had decreased platelet aggregation and increased bleeding time (8,9). One of the characteristic features of increased ω3 fatty acid availability is a reduction in the content of arachidonic acid in platelet membrane phospholipids, thus decreasing the amount of substrate available for eicosanoid synthesis (10-14). Oral products containing ω3 acids have not been associated, however, with clinically significant bleeding problems (15). Likewise, in patients undergoing abdominal surgery and receiving for 5 days total parenteral nutrition supplemented or not with ω3 fatty acids, in doses ≤0.2 g/kg body weight, no significant differences between the groups could be found concerning thromboplastin time, fibrinogen, antithrombin III, and platelet counts or function (16). Nevertheless, it was judged mandatory to assess the hemostatic safety of the present approach for the rapid enrichment of cell phospholipids in ω3 acids.

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Abbreviations: FITC, fluorescein isothiocyanate; MCT:LCT, medium-chain fatty acid triglyceride:soybean triglyceride; MCT:FO, medium-chain fatty acid triglyceride:fish oil; PE, phycoerythrin

Key words: medium-chain triglyceride:fish oil emulsion, long-chain polyunsaturated ω3 fatty acids, clinical trial, hemostatic tests

Materials and methods

As indicated elsewhere (7), 12 healthy male subjects, aged 29.3±1.5 years, volunteered for this study. All subjects received complete information on the potential risks and purpose of the trial, and signed an informed consent before the study. Its design was approved by the Ethics Committee of Erasmus Hospital (Brussels Free University School of Medicine, Brussels, Belgium).

The test preparation (MCT:FO) was a 20% (w/v) emulsion containing 80% of medium-chain fatty acid triglycerides (MCT) and 20% of fish oil (FO), manufactured for the present study by B. Braun AG (Melsungen, Germany). The control preparation (MCT:LCT) was a 20% (w/v) emulsion containing equal amounts (w) of MCT lipids and soybean triglycerides, currently used for parental nutrition (Medialipid®, B. Braun).

After an overnight fast, the subjects drank 200 ml of a carbohydrate solution (Nutricia preOp; Nutricia, Zoetermeer, The Netherlands) 90 min before the lipid injection. One and 8 h after the bolus injection (50 ml) of the lipid emulsions through an intravenous catheter inserted in one arm and at 8,00 a.m. on days 2, 3 and 8 thereafter, blood samples were collected for the present measurements. There was an 8-week wash out period between the injections of each preparation.

The following determinations were made. The PFA-100® device (Dade Behring; Deerfield, IL, USA) was used to measure platelet function in whole citrated blood at high shear conditions. The method determines the time to occlusion of an aperture in a membrane coated with collagen and the weak platelet activator epinephrine (Col-epinephrine) which is particularly sensitive to interference on the cyclo-oxygenase (COX) pathway of platelet aggregation or in a membrane coated with collagen and the strong, COX independent, platelet activator ADP (Col-ADP).

For detection of platelet reactivity by flow cytometry, three combinations of markers were used. Each combination included anti-CD41-Per-CP, a monoclonal antibody that targets all platelets, together with two other markers, i.e. either PAC-1- fluorescein isothiocyanate (FITC) and CD62p-phycoerythrin (PE), fibrinogen-FITC and CD42b-PE, or irrelevant monoclonal antibodies from the same isotype FITC and PE as control. All monoclonal antibodies were from Becton Dickinson/Pharmingen (San Diego, CA, USA), and fibrinogen-FITC from Dako (Gloscrup, Denmark). Whole blood (5 μl) containing sodium citrate was placed in poly-propylene Eppendorf tubes (1.8 ml) together with 9 μl of a combination of each marker (3 μl each, except fibrinogen-FITC in which case 1 μl of the marker was diluted with 2 μl PBS). For each of the three marker combinations, the four following conditions were examined: unstimulated blood (addition of 5 μl PBS), blood stimulated with collagen (addition of 5 μl Chrono-PAR #385; Chrono-Log Corp., Havertown, PA, USA; 1/250 final dilution), blood stimulated with ADP (Roche Diagnostics, Basel, Switzerland; 4 μM final concentration), and blood stimulated with Thrombin Receptor Activator Peptide (TRAP-6; Bachem Biochemical GmbH, Heidelberg, Germany; 65 μM final concentration). After reacting for 15 min at room temperature, the sample was fixed by adding 1.0 ml of 1% formaldehyde. Labelled platelets were then subjected to flow cytometry using a FACSCalibur flow cytometer (Becton-Dickinson). Platelets were gated in a dot plot by FITC-labeled CD41 and Side Scatter (SSC in logarithmic scale). At least 10,000 gated cells were acquired. The percentages of cells recorded with the CD62p and PAC-1 markers were corrected for the paired value found with the isotope PE and FITC control, respectively.

All results are presented as mean values (± SEM) together with the number of individual observations (n). The statistical significance of differences between mean values was assessed by use of Student's t-test.

Results

ADP-test. In the ADP-test, the basal occlusion time, before injection of the lipid emulsion, was not significantly different ($p>0.2$ in the MCT:LCT series (98.2±5.2 s; n=12) and MCT:FO series (89.5±4.5 s; n=12), with an overall mean value of 93.8±3.5 s (n=24). As judged from the latter value, the lower and upper limits of the 95% confidence interval, taken as the mean value±SD.$t_{0.05}$ (d.f. = 23) would be 58.6 and 129.0 s, as compared to 58.0 and 142.0 in the norms of our hospital.

The mean occlusion time after injection of the MCT:LCT emulsion was always lower than mean basal value (Fig. 1, upper left panel). As judged from paired comparison, such a difference was highly significant ($p<0.005$ or less) eight hours and eight days after injection of the MCT:LCT emulsion (Table I). Over the entire period of observation (one hour to eight days after administration of the emulsion), the mean occlusion time, as derived from 4-5 measurements in each subject, represented 88.3±4.3% (n=12; $p<0.03$) of paired basal value.

An essentially comparable situation prevailed in the MCT:FO series. First, one hour after injection of the MCT:FO emulsion, the mean occlusion time, expressed relative to period basal value, was not significantly different ($p>0.7$) in the MCT:LCT series (94.2±4.5%; n=11) and MCT:FO series (95.9±3.2%; n=12). Second, a significant lowering of the occlusion time to 91.1±2.7% (n=12; $p>0.02$) of paired basal value was observed eight hours after injection of the MCT:FO emulsion, in the same manner as already mentioned after injection of the MCT:LCT emulsion. Last, over the entire period of observation, the mean occlusion time represented, after injection of the MCT:FO emulsion, 95.1±2.0% (n=12; $p<0.04$) of paired basal value. The latter percentage was not significantly different ($p>0.1$) from that recorded after injection of the MCT:LCT emulsion. The mean individual percentage computed after injection of the MCT:FO emulsion averaged 109.0±5.2% (n=12; $p>0.1$) of the paired percentage found in the same subject after injection of the MCT:LCT emulsion.

A significant difference between the MCT:LCT and MCT:FO series was only reached by pooling together all the measurements (expressed relative to paired basal value) made in each of these two series. In this case, the values averaged 87.2±2.1% (n=56; $p<0.001$ versus unity) in the MCT:LCT series, as distinct ($p<0.006$) from 94.6±1.5% (n=60; $p<0.001$ versus unity) in the MCT:FO series. Thus, at the most these was a trend towards a lesser decrease of the occlusion time after injection of the MCT:FO, as distinct from MCT:LCT, emulsion.

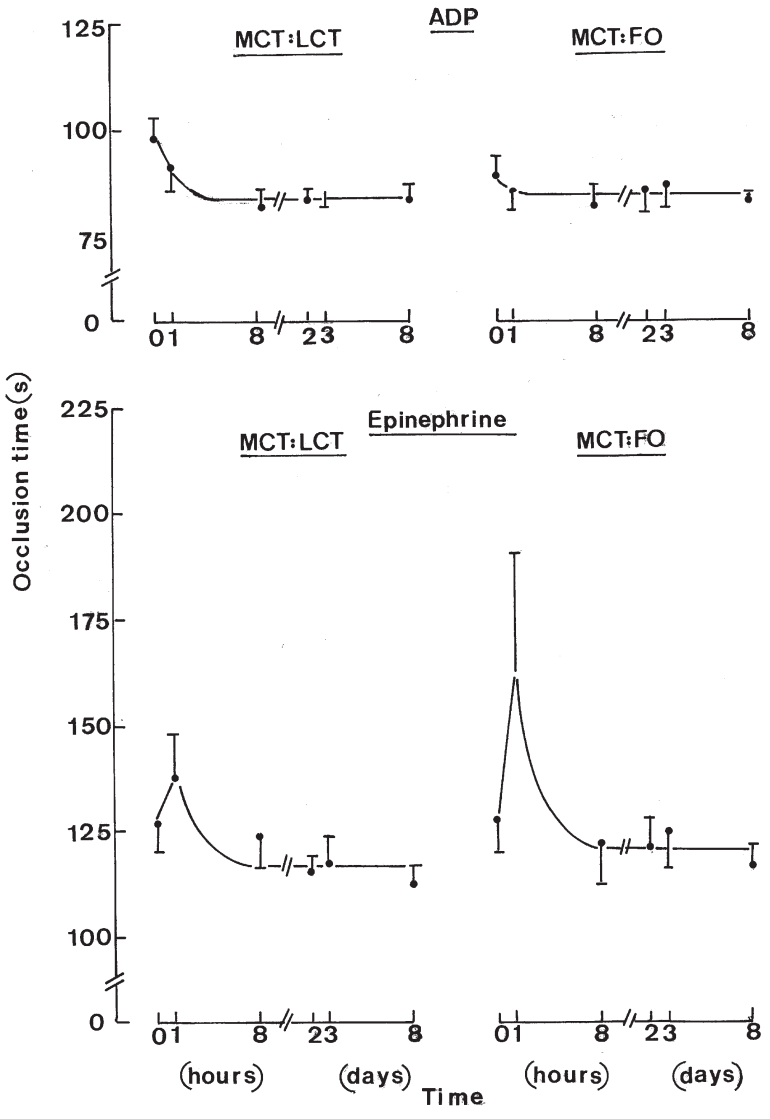

Figure 1. Occlusion time in the coagulation ADP-test (upper panels) and epinephrine-test (lower panels) in subjects injected at time zero with an MCT:LCT (left panels) or MCT:FO (right panels) emulsion. Mean value (± SEM) refer to 11-12 individual determinations.

Table I. Occlusion time in the ADP and epinephrine-test before (basal) and after injection of the MCT:LCT or MCT:FO emulsion.

Test	ADP		Epinephrine	
Emulsion	MCT:LCT	MCT:FO	MCT:LCT	MCT:FO
Basal	100.0[a]	100.0	100.0	100.0
Hour 1	94.2±4.5 (11)	95.9±3.2 (12)	108.2±4.2 (11)	117.0±10.7 (12)
Hour 8	83.8±3.7 (11)	91.1±2.7 (12)	98.1±5.2 (12)	93.8±4.5 (11)
Day 2	87.6±5.5 (12)	95.9±2.6 (12)	92.2±5.7 (12)	93.5±5.1 (12)
Day 3	88.4±6.1 (12)	97.3±4.0 (12)	92.1±6.1 (12)	97.1±6.5 (12)
Day 8	82.0±3.3 (10)	93.1±3.9 (12)	89.0±5.5 (11)	90.7±4.3 (12)

[a]All results are expressed relative to paired basal value.

Epinephrine-test. In the epinephrine-test, the occlusion time averaged, before the injection of a lipid emulsion, 126.8±5.0 s (n=24). It was not significantly different (p>0.9) in the MCT:LCT series (125.4±7.1 s; n=12) and MCT:FO series (127.2±7.5 s; n=12). The upper limit of the 95% confidence interval (see above) amounted to 177.9 s, a value in fair

Table II. Mean values (± SEM) of the median fluorescence intensity for the [CS]42b, [CD]62p and fibrinogen markers.

Time		Min -10	Min 0	Hour 1	Hour 8	Day 2	Day 3	Day 8
[CD]42b								
Control	MCT:LCT	168.6±2.4	166.9±1.4	167.5±1.3	165.4±3.3	166.4±1.5	155.9±4.1	165.6±2.1
	MCT:FO	160.0±4.7	157.3±4.2	163.7±4.0	172.6±2.4	158.9±3.8	165.6±1.7	165.0±2.0
ADP	MCT:LCT	168.3±1.6	161.3±2.7	164.3±1.8	165.4±2.6	162.2±2.4	161.3±3.6	166.6±1.8
	MCT:FO	161.1±3.7	160.0±3.6	162.1±3.7	169.2±4.2	163.0±3.4	165.3±1.6	164.0±1.0
Collagen	MCT:LCT	172.2±1.6	168.3±2.8	165.9±2.7	168.9±2.0	164.7±2.5	165.7±4.5	168.3±2.0
	MCT:FO	168.0±3.5	164.6±3.7	164.2±3.6	170.2±5.9	163.9±3.1	170.7±2.0	169.0±1.1
TRAP	MCT:LCT	160.9±1.6	158.3±1.5	161.9±1.8	161.2±1.4	158.6±1.2	156.8±2.8	161.1±1.9
	MCT:FO	160.5±2.3	157.1±1.6	162.7±3.7	167.4±1.6	158.4±3.4	161.0±3.1	162.4±2.2
[CD]62p								
Control	MCT:LCT	112.7±4.0	111.6±3.0	114.0±5.6	116.8±3.2	114.2±5.7	111.0±2.3	122.1±3.8
	MCT:FO	116.1±4.4	111.8±2.2	117.4±5.1	116.6±3.5	114.5±2.9	118.4±2.8	124.6±7.0
ADP	MCT:LCT	153.4±3.6	151.7±2.9	146.0±3.4	146.8±4.1	158.1±2.4	153.3±4.0	151.3±4.4
	MCT:FO	158.6±3.7	148.0±4.6	144.2±3.6	146.7±2.7	150.1±3.3	155.9±1.1	153.8±3.0
Collagen	MCT:LCT	144.6±5.3	143.0±4.5	141.8±5.9	145.4±3.3	151.8±3.1	147.0±5.3	147.9±4.4
	MCT:FO	147.9±5.1	142.7±4.4	142.9±3.1	148.7±5.0	150.1±2.6	151.7±2.2	150.1±2.9
TRAP	MCT:LCT	181.6±1.6	177.1±1.4	175.4±1.3	176.6±2.1	180.1±1.0	175.1±3.0	176.4±2.4
	MCT:FO	177.0±4.3	175.9±3.0	176.0±2.8	182.9±1.2	172.5±4.1	178.8±0.7	177.7±1.7
Fibrinogen								
Control	MCT:LCT	84.7±2.3	82.7±2.9	81.5±3.5	98.9±8.8	82.4±3.9	85.4±2.9	89.6±0.7
	MCT:FO	88.5±1.4	88.3±1.3	88.2±1.0	90.8±2.1	89.3±2.9	95.8±6.8	107.1±7.7
ADP	MCT:LCT	120.9±3.1	116.4±3.1	119.8±5.5	118.7±4.0	125.6±3.7	140.3±6.3	130.9±6.6
	MCT:FO	121.3±7.8	112.7±4.7	121.4±6.1	119.3±6.4	132.9±4.8	130.3±5.0	132.4±7.4
Collagen	MCT:LCT	98.9±3.7	93.4±4.3	95.7±7.8	105.3±6.8	104.8±4.2	124.4±9.6	120.9±6.9
	MCT:FO	104.3±6.3	104.2±4.6	104.6±5.4	102.1±7.4	111.8±4.3	116.7±5.8	129.7±9.1
TRAP	MCT:LCT	128.1±3.0	126.1±3.1	137.8±5.4	134.2±4.2	133.9±3.1	144.3±6.6	139.4±5.5
	MCT:FO	134.3±9.8	132.8±5.9	132.0±6.2	137.6±7.4	143.8±7.1	134.0±8.0	150.8±7.9

Mean values refer to 7-9 individual measurements.

agreement with that otherwise considered as the upper limit for normal subjects (i.e. 181.0 s).

In the MCT:LCT series, one subject displayed an abnormally high value (182 s) before injection of the emulsion. In this subject, however, the subsequent increment (Δ) in occlusion time one hour ($\Delta = 26$ s) and eight hours ($\Delta = 18$ s) after injection of the MCT:LCT emulsion remained well below the difference between the upper limit of the 95% confidence interval and corresponding mean value, as established before the injection of a lipid emulsion, such a difference amounting to 51.1 s. In the MCT:LCT series, all other measurements remained well below the upper limit of such a 95% confidence interval. Moreover, even one hour after injection of the MCT:LCT emulsion, the occlusion time averaged 108.2±4.2% (n=11) of paired basal value and, as such, failed to be significantly different ($p>0.05$) from such a basal value.

The situation was not vastly different in the MCT:FO series. Nevertheless, one hour to three days after injection of the MCT:FO emulsion, an abnormally high occlusion time was recorded in four out of twelve subjects, with a mean value from these four measurements of 284.0±44.0 s (range 201 to 360 s). The mean peak value in the epinephrine-test was reached one hour after injection of the MCT:FO emulsion and averaged 117.0±10.7% (n=12) of paired basal value. It was not significantly different, however, from such a basal value ($p>0.1$). Sixty minutes after injection of the MCT:FO emulsion, only two subjects displayed an abnormally high occlusion time averaging 218.2±0.0% (n=2) of paired basal value, as distinct ($p<0.005$) from 109.2±5.1% (n=2) in the

Table III. Mean values (± SEM) of the cell percentages for the [CD]62p, fibrinogen and PAC-1 markers.

Time		Min -10	Min 0	Hour 1	Hour 8	Day 2	Day 3	Day 8
[CD]62p								
Control	MCT:LCT	10.1±8.8	3.4±1.0	2.5±1.2	3.3±2.1	5.1±1.6	4.3±1.3	7.1±3.5
	MCT:FO	1.2±1.0	1.6±0.8	2.2±1.0	1.3±0.8	4.6±2.4	2.8±1.2	15.2±8.7
ADP	MCT:LCT	64.7 ±2.3	59.1±1.9	61.5±3.6	50.4±3.2	72.4±2.8	61.2±4.6	50.6±5.2
	MCT:FO	64.4±5.1	58.0±2.4	48.6±3.1	42.1±3.8	38.5±4.3	63.9±2.0	61.3±2.6
Collagen	MCT:LCT	32.7±5.2	27.0±6.0	23.8±5.7	21.9±5.3	53.0±5.7	35.1±8.3	37.3±4.6
	MCT:FO	26.0±6.3	22.0±5.4	14.5±3.2	15.5±4.8	31.9±7.2	38.5±3.1	44.4±3.5
TRAP	MCT:LCT	78.6±3.9	74.5±3.0	77.2±3.9	68.0±3.3	77.5±4.5	71.6±3.5	64.0±4.2
	MCT:FO	68.6±4.4	73.0±2.9	72.5±3.2	65.7±3.4	69.4±3.8	70.4±3.6	65.1±4.5
Fibrinogen								
Control	MCT:LCT	6.7±1.0	8.0±0.8	7.0±1.2	9.6±1.3	8.4±0.9	9.4±1.1	8.6±0.7
	MCT:FO	10.3±1.3	11.4±2.0	10.6±1.4	10.2±0.6	9.8±0.7	9.8±1.3	23.1±7.3
ADP	MCT:LCT	32.1±3.5	31.1±2.3	34.5±6.3	30.2±3.5	42.9±3.0	51.9±5.6	42.7±5.5
	MCT:FO	39.3±4.6	33.6±4.1	39.0±3.8	27.1±3.7	47.9±4.3	45.3±4.6	46.5±5.2
Collagen	MCT:LCT	22.7±4.3	17.4±2.7	20.9±5.9	25.3±5.4	31.5±5.1	43.0±8.0	38.9±5.2
	MCT:FO	25.9±5.0	28.1±3.6	27.4±3.9	20.0±6.1	31.4±3.7	38.1±3.6	46.1±6.8
TRAP	MCT:LCT	47.6±3.7	48.8±3.4	55.0±6.3	52.7±3.5	57.8±3.3	61.4±4.8	57.5±4.8
	MCT:FO	50.8±6.6	47.6±4.8	53.9±4.3	53.9±4.5	61.6±5.0	50.1±5.5	62.1±5.2
PAC-1								
Control	MCT:LCT	1.3±0.8	4.9±2.7	3.9±2.6	4.0±2.2	5.5±2.2	3.4±1.8	6.7±3.0
	MCT:FO	-0.2±0.6	0.2±0.9	1.0±0.8	0.9±0.8	3.1±2.2	2.7±1.6	6.1±2.3
ADP	MCT:LCT	24.2±6.4	23.9±6.9	29.7±7.4	20.3±3.5	18.3±3.6	12.2±1.7	17.1±3.4
	MCT:FO	14.4±3.3	10.9±2.3	19.9±4.1	24.0±3.2	15.9±3.6	18.8±2.1	15.8±3.1
Collagen	MCT:LCT	26.5±7.0	26.8±5.6	31.9±7.4	21.1±4.1	19.9±4.5	12.8±3.3	23.0±4.2
	MCT:FO	17.3±3.4	16.1±2.6	23.5±2.5	21.1±4.6	18.9±4.0	20.1±3.2	22.1±3.4
TRAP	MCT:LCT	0.6±0.4	0.7±0.6	0.9±0.4	-0.1±0.2	0.7±0.2	0.6±0.4	0.2±0.3
	MCT:FO	-0.1±0.5	-0.4±0.1	0.6±0.4	0.1±0.3	0.1±0.4	0.0±0.2	0.5±0.3

Mean values refer to 7-9 individual measurements.

same two subjects examined one hour after injection of the MCT:LCT emulsion. Incidentally, one of these two subjects was the same as that who displayed an abnormally high basal value before injection of the MCT:LCT emulsion, which, in this subject, was administrated eight weeks before the MCT:FO emulsion. In the ten other subjects examined one hour after injection of a lipid emulsion, the occlusion time, when expressed relative to paired basal value, was not significantly different ($p>0.45$) in the MCT:FO series (103.3±4.4%; n=10) and MCT:LCT series (108.0±5.1%; n=9) and, in both cases, not significantly different from unity ($p>0.1$ or more).

The results so far presented suggest that, in the subjects injected with MCT:FO emulsion, a sizeable increase of the occlusion time in the epinephrine-test was only observed on a few occasions, i.e. in 4 out of 59 measurements. A statistically significant increase of the occlusion time was only reached when pooling together the result obtained in the MCT:LCT and MCT:FO series one hour after the injection of these emulsions. In such a case, the occlusion time in the epinephrine-test indeed averaged 112.7±5.7% (n=23; $p<0.05$ versus unity) of paired basal value. At later times after the injection of the lipid emulsion, however, the mean value for the occlusion time was always lower than the paired basal value, whether in the MCT:LCT or MCT:FO series (Table I). Such a lowering only achieved statistical significance, whether in the MCT:LCT or MCT:FO series, by pooling all available data obtained within each series between eight hours and eight days after injection of the lipid emulsion. These overall mean values indeed averaged, relative to paired basal measurement, 92.9±2.8% (n=47; $p<0.02$) in the MCT:LCT series and 93.8±2.5% (n=47; $p<0.025$) in the MCT:FO series. The latter

two percentages were not significantly different from one another (p>0.8).

When expressing the mean of all measurements (n=4-5) made in each subject after injection of the lipid emulsion relative to paired basal value, percentages averaging 96.5±4.1% (n=12) and 100.3±3.4% (n=12) were found, in the epinephrine-test, in the MCT:LCT and MCT:FO series, respectively. These two percentages were not significantly different from unity (p>0.4 or more). They also were not significantly different from one another, whether judged from group comparison (p>0.4) or paired comparison in each subject (p>0.4). This last analysis reinforces the view that there was no sustained prolongation of the occlusion time after injection of the lipid emulsion. On the contrary, and as already observed in the ADP-test, the trend was towards a lowering of the occlusion time in the epinephrine-test, at least from the eight hours after injection of these emulsions onwards (see above).

Hemostatic tests. The hemostatic variables assessed by fluorescence activated cell sorting were measured in 9 subjects after injection of either the control MCT:LCT emulsion or test MCT:FO emulsion. Six of these subjects were examined on both occasions. All other data were collected at the occasion of the second test.

As indicated in Table II, the median fluorescence intensity mean values for [CD]42b, [CD]62p and fibrinogen in the absence of activator (control) and presence of either ADP, collagen or TRAP failed to differ significantly after injection of the MCT:LCT versus MCT:FO emulsion. As expected, in the case of the [CD]62p and fibrinogen measurements, the mean values displayed the following hierarchy: control < collagen < ADP < TRAP. Such was not the case for the [CD]42b measurements. In the latter case, the majority of the relevant plots displayed a peak-shaped distribution. A more flat shape was only observed in 12.5±7.0 (n=9) and 17.4±7.0 (n=9) percent of the 28 determinations made in the MCT:LCT and MCT:FO series, respectively. The latter two percentages failed to differ significantly from one another (p>0.6).

The cell percentages also failed, as a rule, to differ in the MCT:LCT and MCT:FO series (Table III). A significant difference between these two series was only observed on three occasions ([CD]62p response to collagen at day 2, control fibrinogen values at day 8, and PAC response to ADP at day 3) among 84 comparisons. In the case of the [CD]62p and fibrinogen measurements, the mean values again displayed the following hierarchy: control < collagen < ADP < TRAP.

Discussion

The present findings convincingly document the safety, in terms of hemostasis, of the bolus intravenous injection of the MCT:FO emulsion, as well as MCT:LCT emulsion, under experimental conditions in which the injection of the MCT:FO emulsion results, within 60 min and for at least 24-48 h, in a large increase of the ω3 acid content of phospholipids in both leukocytes and platelets (7).

Two sets of data merit to be mentioned. First, in the PFA-100 device, the mean occlusion time was always lower than basal value from eight hours onwards after injection of a lipid emulsion. This effect, however, only achieved statistical significance when pooling together measurements made at different times after the injection of a lipid emulsion and, moreover, failed to differ significantly with either the MCT:FO or control MCT:LCT emulsion. Second, in the case of the [CD]62p response to collagen, the mean values for the cell percentage were lower one hour to day 2 after injection of the MCT:FO, as distinct from MCT:LCT, emulsion. Such a difference, however, only achieved statistical significance on day 2. At the most, there was thus a trend towards a protective effect of the MCT:FO emulsion on platelet activation by collagen.

A further clinical study (Phase Ib) is now scheduled to be conducted in subjects with moderate cardio-vascular risk factors and reduced heart rate variability.

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References

1. Kris-Etherton PM, Harris WS and Apple LJ: American Heart Association. Nutrition Committee: Fish cosumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106: 2747-2457, 2002.
2. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW Jr, Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St Jeor S, Suttie J, Tribble DL and Bazzarre TL: AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 102: 2284-2299, 2000.
3. Christensen MS, Høy C-E, Becker CC and Redgrave TG: Intestinal absorption and lymphatic transport of eicosapentaenoic (EPA), docosahexaenoic (DHA), and decanoic acids: dependence on intramolecular triacylglycerol structure. Am J Clin Nutr 61: 56-61, 1995.
4. Oliveira FL, Rumsey SC, Schlotzer E, Hansen I, Carpentier YA and Deckelbaum RJ: Triglyceride hydrolysis of soy oil vs fish oil emulsions. J Parent Enteral Nutr 21: 224-229, 1997.
5. Carpentier YA and Hacquebard M: Intravenous lipid emulsions to deliver omega 3 fatty acids. Prostaglandins Leukot Essent Fatty Acids 75: 145-148, 2006.
6. Carpentier YA, Portois L and Hacquebard M: Intravascular metabolism of lipid emulsions containing ω3 fatty acids. Clin Nutr (Suppl) 2: 3-5, 2007.
7. 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.
8. Dyerberg J and Bang HO: Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet II: 433-435, 1979.
9. Hirai A, Terano T, Hamazaki T, Sajiki J, Kondo S, Ozawa A, Fujita T, Miyamoto T, Tamura Y and Kumagai A: The effects of the oral administration of fish oil concentrate on the release and the metabolism of [14C]arachidonic acid and [14C]eicosapentaonic acid by human platelets. Thromb Res 28: 285-298, 1982.
10. Sanders TAB, Vickers M and Haines AP: Effects on blood lipids and haemostasis of a supplement of cod-liver oil rich in eicosapentaenoic and docosahexaenoic acids in healthy young men. Clin Sci 61: 317-324, 1981.

11. Goodknight SH, Harris WS and Connor WE: The effects of dietary omega 3 fatty acids on platelet composition and function: a prospective, controlled study. Blood 58: 880-885, 1981.
12. Von Schacky C, Fisher S and Weber PC: Long-term effects of dietary marine ω-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J Clin Invest 76: 1626-1631, 1985.
13. Siess W, Roth P, Scherer B, Kurzmann I, Bohlig B and Weber PC: Platelet-membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. Lancet I: 441-444, 1980.
14. Thorngren M, Shafi S and Born GV: Delay in primary haemostasis produced by a fish diet without change in local thromboxan A2. Br J Haematol 58: 567-578, 1984.
15. Simopoulos AP: Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 54: 438-463, 1991.
16. Heller AR, Fischer S, Rössel T, Geiger S, Siegert G, Ragaller M, Zimmermann T and Koch T: Impact of n-3 fatty acid supplemented parenteral nutrition on haemostasis patterns after major abdominal surgery. Br J Nutr 87 (Suppl 1): S95-S101, 2002.