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Pancreatic Islet Function in ω3 Fatty Acid–Depleted Rats

Glucose Metabolism and Nutrient-Stimulated Insulin Release

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In order to gain information on the determinism of the perturbation of fuel homeostasis in situations characterized by a depletion in long-chain polyunsaturated ω3 fatty acids (ω3), the metabolic and hormonal status of ω3-depleted rats (second generation) was examined. When required, these rats were injected intravenously 120 min before sacrifice with a novel medium-chain triglyceride–fish oil emulsion able to provoke a rapid and sustained increase of the ω3 content in cell phospholipids. The measurement of plasma glucose, insulin, phospholipid, triglyceride, and unesterified fatty acid concentration indicated modest insulin resistance in the ω3-depleted rats. The plasma triglyceride and phospholipid concentrations were decreased in the ω3-depleted rats with abnormally low contribution of ω3 in both circulating and pancreatic islet lipids. The protein, insulin, and lipid content of the islets, as well as their intracellular and extracellular spaces, were little affected in the ω3-depleted rats. The metabolism of D-glucose in the islets of ω3-depleted rats was characterized by a lesser increase in D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation in response to a given rise in hexose concentration and an abnormally low ratio between D-glucose oxidation and utilization. These abnormalities could be linked to an increased metabolism of endogenous fatty acids with resulting alteration of glucokinase kinetics. The release of insulin evoked by D-glucose, at a close-to-physiological concentration (8.3 m*M*), was increased in the ω3-depleted rats, this being considered as consistent with their insulin resistance. Relative to such a release, that evoked by a further rise in D-glucose concentration or by non-glucidic nutrients was abnormally high in ω3-depleted rats, and restored to a normal level after of the intravenous injection of the ω3-rich medium-chain triglyceride–fish oil emulsion. Because the latter procedure failed to correct the perturbation of D-glucose metabolism in the islets of ω3-depleted rats, it is proposed that the anomalies in the secretory behaviour of islets in terms of their response to an increase in hexose concentration or non-nutrient secretagogues is mainly attributable to alteration in K^+ and Ca^{2+} handling, as indeed recently documented in separate experiments.

Key Words: Long-chain polyunsaturated ω3 fatty acids; pancreatic islets; D-glucose metabolism; insulin secretion.

Introduction

A deficiency in long-chain polyunsaturated ω3 fatty acids presently often prevails in the Western population *(1,2)*. This coincides with qualified perturbations of fuel homeostasis *(3,4)*. With this information in mind, we are presently engaged in an extensive study, based on the comparison between normal and ω3-depleted rats (second generation), on the metabolic and hormonal situation found in vivo in these animals, as well as on selected biochemical, biophysical, and functional variables in muscles, adipocytes, and pancreatic islets examined in vitro *(5–11)*. In all these investigations, advantage is taken of the development of a novel medium-chain triglyceride–fish oil emulsion (MCT:FO; 8:2) recently found to provoke a rapid and sustained increase of the content in long-chain polyunsaturated ω3 fatty acids of cell phospholipids, as documented either in vitro in cultured endothelial cells or in vivo in rat liver cells or human platelets and leukocytes after the bolus intravenous injection of this emulsion *(5,12)*. Thus, the situation found in untreated or saline-injected ω3-depleted rats was compared to that found 60–120 min after the bolus intravenous injection of either the MCT:FO emulsion or a control medium-chain triglyceride–olive oil emulsion (MCT:OO).

The present report provides information on (i) some metabolic and hormonal variables measured in fed animals, including plasma glucose, insulin, phospholipid, triglyceride, and unesterified fatty acid concentration; (ii) the pro-

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Table 1
Metabolic and Hormonal Status of Normal and w3-Depleted Rats

Rats	Normal	NI-ω3	OO-ω3	FO-ω3
Age (wk)	12.0 ± 0.5 (34)	19.9 ± 4.9 (13)	13.0 ± 0.3 (30	13.0 ± 0.3 (30)
Body weight (g)	224.6 ± 5.8 (34)	269.9 ± 6.2 (13)	262.6 ± 2.7 (30)	260.9 ± 3.1 (30)
Plasma D-glucose (mmol/L)	7.03 ± 0.40 (34)	8.40 ± 0.89 (12)	12.91 ± 0.54 (30)	12.15 ± 0.68 (30)
Plasma insulin (ng/mL)	0.93 ± 0.10 (34)	1.39 ± 0.18 (12)	1.55 ± 0.09 (30)	1.73 ± 0.13 (30)
Insulinogenic index (μg/mol)	136 ± 16 (34)	212 ± 50 (12)	125 ± 8 (30)	151 ± 12 (30)
Plasma phospholipids (mg/L)[a]	1,043 ± 26 (4)	843 ± 24 (9)	1068 ± 44 (6)	1029 ± 26 (6)
Plasma triglycerides (mg/L)[a]	1,074 ± 126 (4)	428 ± 21 (8)	622 ± 57 (6)	482 ± 61 (6)
Plasma diglycerides (mg/L)[a]	N.D.[b]	7.18 ± 0.69 (9)	12.55 ± 1.70 (6)	9.89 ± 0.88 (6)
Plasma non-esterified fatty acids (μmol/L)	123 ± 9 (4)	282 ± 26 (8)	398 ± 16 (6)	409 ± 54 (6)
Islet protein content (μg/islet)	0.98 ± 0.08 (14)	1.49 ± 0.31 (12)	1.34 ± 0.27 (12)	1.31 ± 0.19 (12)
Islet insulin content (ng/islet)	19.30 ± 1.46 (15)	18.40 ± 1.79 (12)	20.90 ± 1.05 (12)	17.36 ± 0.93 (12)
Islet insulin/protein ratio (ng/μg)	20.88 ± 1.88 (14)	17.62 ± 2.73 (12)	19.59 ± 2.04 (12)	16.08 ± 2.00 (12)

[a]Expressed as fatty acids.
[b]N.D.: not determined.

tein, insulin, and triglyceride content, as well as lipid fatty acid pattern, of isolated pancreatic islets; (iii) the metabolism of D-glucose in the islets; and (iv) their insulin secretory response to nutrient secretagogues.

Results

Metabolic and Hormonal Status

At comparable age ($p > 0.1$), the ω3-depleted rats displayed a higher body weight ($p < 0.001$) than normal rats (Table 1).

The plasma glucose concentration was slightly, but not significantly ($p > 0.1$), higher in NI-ω3 rats than in normal animals. The plasma insulin concentration, however, was significantly higher ($p < 0.03$) in the NI-ω3 rats than in control animals. The mean insulinogenic index, i.e., the paired ratio between plasma insulin and glucose concentration, was also higher, albeit not quite significantly so ($p < 0.07$) in the NI-ω3 rats than in the control animals. When the ω3-depleted rats were injected with a lipid emulsion, the plasma glucose concentration averaged 12.53 ± 0.43 m*M* ($n = 60$) and, as such, was higher ($p < 0.001$) than in the NI-ω3 rats. As already observed in human subjects *(12)*, such an increase failed to coincide with a significant increase of plasma insulin concentration, when comparing the ω3-depleted rats injected with a lipid emulsion (1.64 ± 0.08 ng/mL; $n = 60$) and the NI-ω3 rats (1.39 ± 0.18 ng/mL; $n = 12$). Actually, the insulinogenic index was slightly but significantly ($p < 0.01$) lower in the ω3-depleted rats injected with a lipid emulsion (135 ± 7 μg/mol; $n = 60$) than in the NI-ω3 rats (212 ± 50 μg/mol; $n = 12$).

In these animals given free access to food, the HOMA (plasma glucose concentration, expressed as m*M*, multiplied by plasma insulin concentration, expressed as ng/mL) averaged 10.95 ± 1.61 ($n = 12$) in NI-ω3 rats, as distinct ($p < 0.06$) from only 7.29 ± 0.94 ($n = 34$) in non-injected normal animals. Such a difference became fully significant ($p < 0.025$) when the calculation was based, as should be the case, on the geometric means. As expected from the increase in gluconeogenesis and, hence, plasma glucose concentration, the HOMA was even higher in ω3-depleted rats injected with either the MCT:OO (20.26 ± 1.51; $n = 30$) or MCT:FO (21.76 ± 2.43; $n = 30$) emulsion.

Table 1 also provides information on the plasma concentration of difference classes of lipids in the normal and ω3-depleted rats. The concentration of phospholipids and triglycerides were significantly higher ($p < 0.001$) in normal rats than in NI-ω3 animals, while a mirror image ($p < 0.005$) prevailed in the case of non-esterified fatty acids. As expected, the plasma concentration of phospholipids, triglycerides, diglycerides, and unesterified fatty acids were all higher ($p < 0.01$ or less) in OO-ω3 than NI-ω3 rats. Such was also the case ($p < 0.05$ or less) for the plasma phospholipids and unesterified fatty acids in the FO-ω3 rats. The plasma concentration of triglycerides and diglycerides, however, only represented in the FO-ω3 rats 78.2 ± 5.7% ($n = 12$; $p < 0.05$) of the mean corresponding values found in the OO-ω3 rats (100.0 ± 7.8%; $n = 12$). Such a difference is consistent with the knowledge that the fate of the MCT:OO and MCT:FO emulsion, following their intravenous injection, differs in some respects, whether in normal or ω3-depleted rats *(5)*.

Sizable amounts of C20:5ω3, C22:5ω3, and C22:6ω3 were found in the plasma phospholipids and triglycerides of normal rats, averaging, respectively, 4.44 ± 0.33, 11.68 ± 0.78, and 71.86 ± 2.68 mg/L in the phospholipids ($n = 4$) and 6.67 ± 0.64, 6.27 ± 0.65, and 24.08 ± 3.39 mg/L in the triglycerides ($n = 3$). In the ω3-depleted rats, whether injected with a lipid emulsion or not, detectable amounts of C22:5ω3 and C22:6ω3 (but not C20:5ω3) were only found in plasma phospholipids, in which they averaged, respectively, 2.50 ± 0.27 and 9.42 ± 0.24 mg/L ($n = 21$), values

Table 2
Liver Phospholipid Content in Long-Chain Polyunsaturated ω3 Fatty Acids

Rats	Normal	OO-ω3	FO-ω3
C20:5ω3	160.5 ± 15.5 (10)[a]	N.D. (30)[b]	0.8 ± 0.8 (30)
C22:5ω3	338.9 ± 20.0 (10)	34.6 ± 1.2 (30)	40.4 ± 1.6 (30)[d]
C22:6ω3	3,116.8 ± 173.0 (10)	332.1 ± 10.4 (30)	369.8 ± 12.7 (30)[c]

[a]All results are expressed as μg/g liver wet wt.
[b]N.D.: not detected.
[c]$p < 0.03$ and [d]$p < 0.01$ for the difference between OO-ω3 and FO-ω3 rats.

about five to eight times lower ($p < 0.001$) than those found in the normal rats.

The total amount of fatty acids present in the phospholipids of leukocytes was not significantly different ($p > 0.25$) in the OO-ω3 rats (23.0 ± 1.9 μg/mL of blood; $n = 5$) and FO-ω3 rats (25.4 ± 1.0 μg/mL; $n = 5$). No sizable enrichment of such phospholipids in C20:5ω3 could be documented in the FO-ω3 rats, all readings remaining below the limit of detection. The content of leukocyte phospholipids in C22:6ω3 was increased, however, to 88.1 ± 8.2 ng/mL of blood ($n = 5$; $p < 0.001$) in the FO-ω3 rats, as distinct from being below the limit of detection (<7 ng/mL) in the five samples prepared from OO-ω3 rats.

The increase of the cell phospholipid content in long-chain polyunsaturated ω3 fatty acids, as provoked by the injection of the MCT:FO emulsion, is further documented in Table 2, which relates to measurements made in liver extracts. The C20:5ω3 content of hepatic phospholipids remained below the limit of detection in all ω3-depleted animals, except in one FO-ω3 rat. In the OO-ω3 rats, the liver phospholipid content in C22:5ω3 and C22:6ω3 was about 10 times lower than in normal rats ($p < 0.001$). In the FO-ω3 rats, the amount of both C22:5ω3 and C22:6ω3 was significantly higher ($p < 0.03$) than in OO-ω3 rats. Such was also the case ($p < 0.02$) when the results were expressed relative to the total amount of fatty acids present in the liver phospholipids (data not shown). The absolute amounts of C22:5ω3 and C22:6ω3 found in the liver phospholipids of FO-ω3 rats remained, nevertheless, much lower ($p < 0.001$) than those found in normal rats.

Because both the MCT:OO and MCT:FO emulsion contain 4.0 mg/mL all-rac-α-tocopherol, the α-tocopherol content was also measured in the liver of normal rats and ω3-depleted animals injected with one of these two emulsions. Such a content increased ($p < 0.02$ or less) from a mean value of 14 ± 3 ng/mg liver wet wt ($n = 4$) in non-injected normal rats to 92 ± 11 ($n = 28$) and 78 ± 6 ($n = 30$) ng/mg in OO-ω3 and FO-ω3 rats, respectively. The later two values were not significantly different from one another ($p > 0.2$). In platelets and leukocytes, respectively, the α-tocopherol content averaged 63.1 ± 3.8 and 37.8 ± 4.7 μmol per m*M* of phospholipids in the OO-ω3 rats and 65.8 ± 5.7 and 33.8 ± 2.5 μmol per m*M* of phospholipids in the FO-ω3 rats ($n = 4$–5). Once again, there was no significant difference between OO-ω3 and FO-ω3 rats for the latter two variables ($p > 0.4$ or more).

Islet Data

The protein content of the islets was not significantly different in the four groups of rats considered in Table 1. Such was also the case for the islet insulin content expressed relative to paired protein content. The absolute value for the islet insulin content (ng/islet) also failed to differ significantly, except when comparing the OO-ω3 and FO-ω3 rats in which case the value found in the latter animals was significantly lower ($p < 0.025$) than that recorded in the former ones.

The ^{3}HOH and L-[1-^{14}C]glucose distribution spaces were not significantly different ($p > 0.1$ or more) in the islets prepared from OO-ω3 and FO-ω3 rats, with overall mean values of 2.44 ± 0.20 and 0.73 ± 0.11 nL/islet ($n = 33$ in both cases). As judged from these measurements, the extracellular space (expressed relative to the paired ^{3}HOH distribution space) and the intracellular space (taken as the paired difference between the ^{3}HOH and L-[1-^{14}C]glucose spaces) also failed to differ significantly ($p > 0.1$ or more) in these two groups of ω3-depleted rats, with overall mean respective values of 27.9 ± 3.1% ($n = 32$) and 1.78 ± 0.14 nL/islet ($n = 31$).

The triglyceride content of the islets, expressed as glycerol equivalent, averaged 116.8 ± 14.0 pmol/islet ($n = 14$) in normal rats. It was slightly, but not significantly ($p < 0.1$), higher in ω3-depleted rats (155.1 ± 16.5 pmol/islet; $n = 17$). As judged from their weight percentage ($n = 5$ in all cases), the most abundant fatty acids in the islet lipids were C20:4ω6 (27.0 ± 1.1%), C16:0 (23.4 ± 1.4%), C18:0 (19.3 ± 0.4%), C18:2ω6 (12.9 ± 1.5%), and C18:1ω9 (9.0 ± 0.9%). Sizable amounts of long-chain polyunsaturated ω3 fatty acids were detected in the islets of normal rats, yielding a value of 1.15 ± 0.14% ($n = 2$) as the weight percentage for the sum of C20:5ω3 and C22:5ω3. No long-chain polyunsaturated ω3 fatty acids could be detected in the islets from ω3-depleted rats ($n = 3$). The fatty acid pattern here found in the islet lipids of normal rats yielded mean paired C18:0/C16:0

Table 3
Metabolic Data in Islets From Normal and ω3-Depleted Rats

Rats		Normal	NI-ω3 & OO-ω3	FO-ω3
D-[5-^{3}H]glucose conversion to ^{3}HOH				
D-glucose	2.8 mmol/L	36.5 ± 2.6 (23)[a]	53.1 ± 3.3 (27)	41.0 ± 3.4 (15)
	8.3 mmol/L	81.3 ± 6.9 (23)	101.4 ± 9.6 (29)	82.6 ± 8.0 (18)
	16.7 mmol/L	133.8 ± 8.7 (24)	122.4 ± 16.9 (28)	122.6 ± 12.5 (16)
D-[U-^{14}C]glucose conversion to $^{14}CO_2$				
D-glucose	2.8 mmol/L	8.3 ± 0.6 (25)	8.5 ± 0.6 (30)	8.9 ± 0.9 (17)
	8.3 mmol/L	23.6 ± 1.4 (25)	20.7 ± 1.8 (29)	18.3 ± 2.3 (19)
	16.7 mmol/L	45.3 ± 2.5 (24)	28.8 ± 3.6 (25)	25.4 ± 3.0 (15)

[a]All results are expressed as pmol/islet over 90 min incubation.

(0.96 ± 0.20), C18:1ω9/C18:0 (0.36 ± 0.04), and C20:4ω6/C18:2ω6 (2.11 ± 0.33) ratios ($n = 2$ in all cases) comparable to those reported in a prior study *(13)*, with a correlation coefficient between the two series of observation of 0.975. In this respect, the present values averaged 92.7 ± 12.6% ($n = 6$; $p > 0.5$ vs unity) of the mean corresponding values found in the prior report *(13)*.

Metabolic Data

The primary data relative to the conversion of D-[5-^{3}H]glucose to ^{3}HOH (glucose utilization) and D-[U-^{14}C]glucose to $^{14}CO_2$ (glucose oxidation) in islets from normal and ω3-depleted rats are listed in Table 3. Two major anomalies of D-glucose metabolism were observed in the islets of ω3-depleted rats. The first of these anomalies concern the metabolic response to increasing concentrations of extracellular D-glucose concentration.

In the low range of D-glucose concentrations, the mean 8.3/2.8 m*M* ratios for ^{3}HOH and $^{14}CO_2$ production averaged, respectively, 1.97 ± 0.18 (d.f. = 85) and 2.27 ± 0.20 (d.f. = 89) in ω3-depleted rats, as compared to 2.23 ± 0.25 (d.f. = 44) and 2.86 ± 0.27 (d.f. = 48) in normal rats (Fig. 1). The values found in the ω3-depleted rats thus represented only 83.8 ± 5.3% (d.f. = 174; $p < 0.075$) of those recorded in control rats (100.0 ± 7.1%; d.f. = 92). For the two variables under consideration, there was no significant difference between NI-ω3, OO-ω3, and FO-ω3 rats. Incidentally, the results so far mentioned also document, in both normal and ω3-depleted rats, a preferential stimulation of D-[U-^{14}C]glucose oxidation relative to D-[5-^{3}H]glucose utilization in response to the rise in hexose concentration from 2.8 to 8.3 m*M*. The paired ratio between $^{14}CO_2$ output and ^{3}HOH production indeed averaged, at 8.3 m*M* D-glucose, 132.7 ± 5.5% ($n = 69$; $p < 0.001$) of the mean corresponding value found in the same type of rats at 2.8 m*M* D-glucose (100.0 ± 3.4%; $n = 65$).

In response to a further rise in D-glucose concentration from 8.3 to 16.7 m*M*, the relative magnitude of the resulting increase in D-glucose utilization and oxidation was again less pronounced in islets from ω3-depleted rats than in those

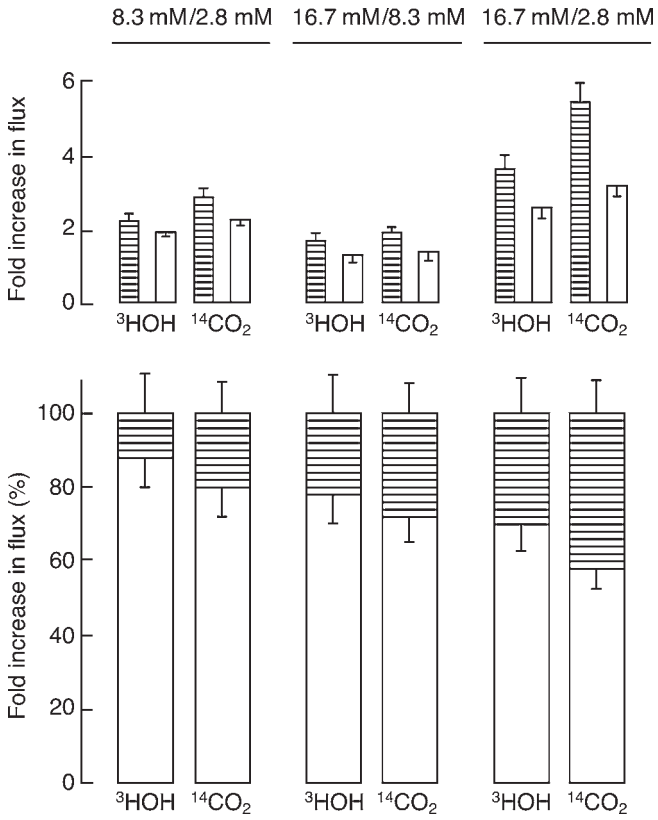

Fig. 1. The fold increase in ^{3}HOH generation from D-[5-^{3}H]glucose and $^{14}CO_2$ generation for D-[U-^{14}C]glucose in response to increases in extracellular D-glucose concentration in islets from normal rats (hatched columns) or ω3-depleted animals (open columns) are expressed either in absolute terms (upper panel) or relative to that found in normal rats (lower panel). Mean values (± SEM) refer to d.f. of 44–89.

from normal rats. The 16.7/8.3 m*M* ratios for ^{3}HOH and $^{14}CO_2$ production averaged, respectively, 1.18 ± 0.19 (d.f. = 55) and 1.38 ± 0.17 (d.f. = 52) in the NI-ω3 and OO-ω3 rats, as compared to 1.65 ± 0.17 (d.f. = 45) and 1.92 ± 0.16 (d.f. = 47) in normal rats. Thus, the values found in the NI-ω3 and OO-ω3 rats only represented 72.3 ± 7.2% (d.f. = 107; $p < 0.01$) of those recorded in normal rats (100.0 ±

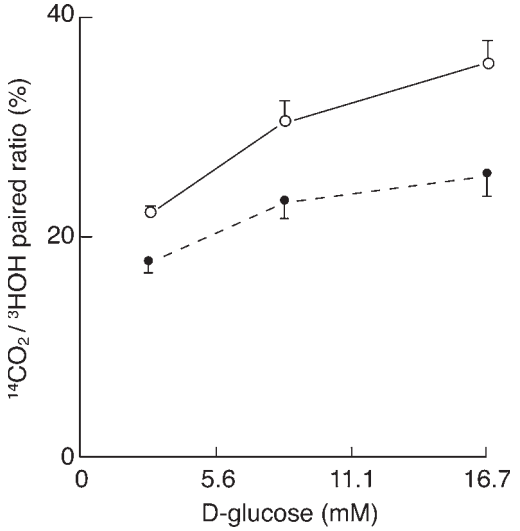

Fig. 2. Paired ratio between $^{14}CO_2$ generation from D-[U-^{14}C] glucose and ^{3}HOH generation from D-[5-^{3}H]glucose in islets from normal rats (open circles and solid line) or ω3-depleted animals (closed circles and dashed line) incubated at increasing concentrations of D-glucose. Mean values (± SEM) refer to 22–47 separate determinations.

6.7%; d.f. = 92). In this respect, the results obtained in FO-ω3 rats (81.3 ± 9.1%; d.f. = 64) were not significantly different ($p > 0.4$) from those found in NI-ω3 and OO-ω3 rats, but also failed to differ significantly ($p < 0.09$) from those recorded in normal rats. Once again, the rise in D-glucose concentration from 8.3 to 16.7 m*M* increased $^{14}CO_2$ output relative to ^{3}HOH generation, in both normal and ω3-depleted rats, the 16.7/8.3 m*M* ratio for this variable ($^{14}CO_2/^3$HOH) averaging 111.1 ± 5.7% (d.f. = 126; $p < 0.06$ vs unity).

The lesser relative increase in D-glucose metabolism at increasing concentrations of the hexose found in the islets of ω3-depleted rats was further documented by computing the 16.7/2.8 m*M* ratio for ^{3}HOH and $^{14}CO_2$ generation (Fig. 1). It averaged, in the ω3-depleted rats, 2.57 ± 0.29 (d.f. = 80) as distinct ($p < 0.025$) from a normal value of 3.67 ± 0.35 (d.f. = 45) for ^{3}HOH generation, and 3.19 ± 0.31 (d.f. = 81) as distinct ($p < 0.001$) from 5.48 ± 0.49 (d.f. = 47) for $^{14}CO_2$ production.

The findings illustrated in Fig. 1 reveal two further features. First, whether in the case of ^{3}HOH or $^{14}CO_2$ production, the difference between normal and ω3-depleted rats always became progressively more pronounced, in relative terms, as the two concentrations of D-glucose under consideration moved from 8.3/2.8 m*M* to 16.7/8.3 m*M* and 16.7/2.8 m*M*. Thus, expressed relative to the values found in normal rats, those recorded in the ω3-depleted animals represented, in the latter two cases, 90.3 ± 6.7% (d.f. = 167) and 75.7 ± 5.6% (d.f. = 161; $p < 0.01$) of the readings made in the former case (100.0 ± 6.3%; d.f. = 174). This could point to altered kinetics of glucokinase in the islets of ω3-depleted rats. Second, the difference between normal and ω3-depleted rats was always more pronounced for $^{14}CO_2$ output than ^{3}HOH production, suggesting a possible impairment in the ω3-depleted rats of the preferential stimulation of D-glucose oxidation relative to D-glucose utilization at increasing concentrations of the hexose.

The values for the paired ratio between $^{14}CO_2$ output and ^{3}HOH generation were, therefore, further considered. As illustrated in Fig. 2, the mean values for such a ratio in the islets from ω3-depleted rats were always lower ($p < 0.005$ or less) than the corresponding values found at the same D-glucose concentration in islets from normal rats. In this respect, there was no significant difference between NI-ω3 and OO-ω3 rats, on the one hand, and FO-ω3 rats, on the other hand. The ratios between the mean values for D-[U-^{14}C]glucose oxidation relative to D-[5-^{3}H]glucose utilization recorded at different D-glucose concentrations (8.3/2.8 m*M*, 16.7/8.3 m*M*, and 16.7/2.8 m*M*) were not significantly lower, however, in ω3-depleted rats than in control animals, the results obtained in the former rats averaging 91.9 ± 4.3% (d.f. = 256) of the corresponding values found in normal animals (100.0 ± 3.9%; d.f. = 130).

Secretory Data

The release of insulin evoked over 90 min incubation by 8.3 m*M* D-glucose, which was measured in each experiment, was virtually identical in NI-ω3 rats, OO-ω3 rats and FO-ω3 rats and, in all three cases, significantly higher ($p < 0.01$ or less) than that found in islets from normal rats (Table 4).

Relative to the mean output of insulin found at 8.3 m*M* D-glucose, that measured within the same experiment(s) at 2.8 m*M* D-glucose was higher ($p < 0.001$) in NI-ω3 rats than in normal rats. This lesser secretory responsiveness to the increase in D-glucose concentration from 2.8 to 8.3 m*M* in the NI-ω3 rats was not corrected by prior injection of the MCT:FO emulsion. On the contrary, expressed in the same manner, the release of insulin found at 2.8 m*M* D-glucose was twice as high ($p < 0.02$) in the FO-ω3 rats than in the OO-ω3 rats.

A somewhat different situation prevailed, however, when comparing the release of insulin provoked by 8.3 m*M* D-glucose in the absence vs presence of the organic calcium antagonist verapamil (20 μM). Indeed, in such a case, the release of insulin found in the presence of verapamil, expressed relative to that recorded in its absence, was lower ($p < 0.005$) in the NI-ω3 rats than in the normal rats. Likewise, the output of insulin found in the presence of verapamil and always expressed relative to that recorded in its absence, was lower ($p < 0.05$) in the OO-ω3 rats than in the FO-ω3 rats. Thus, in respect to the effect of verapamil upon insulin release provoked by 8.3 m*M* D-glucose, the comparison between normal rats and NI-ω3 rats, yielded a multiplication factor (normal/NI-ω3 rats) of 3.67 ± 0.78 (d.f. = 36), not significantly different ($p > 0.1$) from that found (2.17 ± 0.55; d.f. = 21) when comparing FO-ω3 rats to OO-ω3 rats. In other words, the prior injection of the MCT:FO emulsion, while

Table 4
Comparison Between the Secretory Response to D-Glucose and Verapamil in Islets From Normal, NI-ω3, OO-ω3 and FO-ω3 Rats

Rats	Normal	NI-ω3	OO-ω3	FO-ω3
Absolute value (μU/islet per 90 min)				
8.3 mmol/L D-glucose	91.6 ± 4.2 (58)	138.0 ± 10.5 (38)$^{\varepsilon}$	139.0 ± 15.7 (50)	136.5 ± 17.0 (53)
Relative values (%)				
8.3 mmol/L D-glucose	100.0 ± 7.9 (19)	100.0 ± 9.1 (19)	100.0 ± 17.3 (13)	100.0 ± 6.0 (20)
2.8 mmol/L D-glucose	26.2 ± 2.3 (16)^{e}	36.4 ± 1.6 (19)$^{e,\varepsilon}$	23.5 ± 4.9 (16)^{e}	45.6 ± 7.6 (15)$^{e,\beta}$
8.3 mmol/L D-glucose	100.0 ± 5.5 (20)	100.0 ± 9.5 (18)	100.0 ± 5.5 (10)	100.0 ± 8.1 (15)
8.3 mmol/L D-glucose + 20 μmol/L verapamil	39.9 ± 7.8 (20)^{e}	10.9 ± 2.5 (18)$^{e,\delta}$	10.8 ± 1.8 (11)^{e}	23.5 ± 5.5 (12)$^{e,\alpha}$
8.3 mmol/L D-glucose	100.0 ± 7.9 (19)	100.0 ± 9.1 (19)	100.0 ± 22.4 (18)	100.0 ± 9.9 (17)
16.7 mmol/L D-glucose	147.0 ± 9.8 (20)^{e}	191.3 ± 9.8 (19)$^{e,\gamma}$	206.6 ± 7.6 (22)^{e}	155.6 ± 9.5 (21)$^{e,\varepsilon}$

α: $p < 0.05$; *b*: $p < 0.02$; γ: $p < 0.01$; δ: $p < 0.005$ and *e* and ε: $p < 0.001$ for the difference between the first and second line in each set of results (e) and for that between either normal and NI-ω3 rats or OO-ω3 and FO-ω3 rats (α, β, γ, δ, ε).

Table 5
Comparison Between the Insulin Secretory Response to D-Glucose and Non-Glucidic Nutrients in Islets From Normal, OO-ω3, and FO-ω3 Rats

Rats	Normal	OO-ω3	FO-ω3
Relative values (%)			
8.3 mmol/L D-glucose	100.0 ± 7.9 (19)	100.0 ± 7.5 (16)	100.0 ± 9.9 (17)
10.0 mmol/L 2-ketoisocaproate	60.1 ± 3.5 (20)$^{e,\varepsilon}$	129.7 ± 10.2 (24)^{a}	86.5 ± 6.6 (18)$^{\delta}$
8.3 mmol/L D-glucose	100.0 ± 7.9 (19)	100.0 ± 9.4 (12)	100.0 ± 9.9 (17)
20.0 mmol/L L-leucine	33.1 ± 2.4 (17)$^{e,\gamma}$	70.6 ± 12.8 (17)	58.4 ± 3.9 (22)^{e}
8.3 mmol/L D-glucose	100.0 ± 7.9 (19)	100.0 ± 9.4 (12)	100.0 ± 9.9 (17)
20.0 mmol/L L-leucine + 1.0 mmol/L L-glutamine	57.0 ± 3.0 (19)$^{e,\gamma}$	157.5 ± 38.1 (17)	76.2 ± 6.7 (22)$^{a,\beta}$
2.8 mmol/L D-glucose	100.0 ± 8.7 (16)	100.0 ± 25.5 (16)	100.0 ± 10.0 (15)
10.0 mmol/L 2-ketoisocaproate	229.2 ± 13.3 (20)$^{e,\varepsilon}$	405.4 ± 37.0 (24)^{e}	220.1 ± 19.5 (18)$^{e,\varepsilon}$
2.8 mmol/L D-glucose	100.0 ± 8.7 (16)	100.0 ± 25.5 (16)	100.0 ± 10.0 (15)
20.0 mmol/L L-leucine	126.2 ± 9.0 (17)$^{a,\beta}$	272.8 ± 54.0 (17)^{c}	177.1 ± 25.4 (22)^{b}
2.8 mmol/L D-glucose	100.0 ± 8.7 (16)	100.0 ± 25.5 (16)	100.0 ± 10.0 (15)
20.0 mmol/L L-leucine + 1.0 mmol/l L-glutamine	217.5 ± 11.5 (19)$^{e,\beta}$	617.7 ± 156.8 (17)^{d}	218.8 ± 31.4 (22)$^{d,\gamma}$
20.0 mmol/L L-leucine	100.0 ± 7.1 (17)	100.0 ± 9.7 (17)	100.0 ± 4.1 (22)
20.0 mmol/L L-leucine + 1.0 mmol/L L-glutamine	172.3 ± 9.1 (19)^{e}	198.7 ± 29.9 (17)^{d}	134.6 ± 12.8 (22)$^{b,\alpha}$

a and α: $p < 0.05$; *b* and β: $p < 0.025$; *c* and γ: $p < 0.01$; *d* and δ: $p < 0.005$ and *e* and ε: $p < 0.001$ for the difference between the first and second line in each set of results (a, b, c, d, e) and that between OO-ω3 and either normal or FO-ω3 rats (α, β, γ, δ, ε).

failing to correct the decreased responsiveness to a rise in D-glucose concentration from 2.8 to 8.3 m*M* otherwise found in ω3-depleted animals, normalized the functional behavior of the islets from ω3-depleted rats in terms of the inhibitory action of verapamil upon the secretory response to D-glucose (8.3 m*M*).

A comparable normalization was found in response to a rise in D-glucose concentration from 8.3 to 16.7 m*M* (Table 4). Relative to the output of insulin found within the same experiment(s) at 8.3 m*M* D-glucose, that measured at 16.7 m*M* D-glucose was higher ($p < 0.01$) in the NI-ω3 rats than in normal rats. Likewise, it was higher ($p < 0.001$) in OO-ω3 rats than in FO-ω3 rats. The NI-ω3/normal multiplication factor (1.30 ± 0.09; d.f. = 37) was virtually identical to the OO-ω3/FO-ω3 multiplication factor (1.33 ± 0.08; d.f. = 41).

When the release of insulin provoked by non-glucidic nutrients (10.0 m*M* 2-ketoisocaproate, 20.0 m*M* L-leucine in the absence or presence of 1.0 m*M* L-glutamine) was compared to that found, within the same experiment(s), at 8.3 m*M* D-glucose, the mean results obtained in OO-ω3 rats were again always higher than in FO-ω3 rats (Table 5, upper panel). In the latter rats, they averaged 65.9 ± 3.4% ($n = 62$; $p < 0.001$) of the corresponding values found in the OO-ω3 rats (100.0 ± 9.3%; $n = 58$). A comparable situation prevailed when the secretory response to the non-glucidic

nutrients was expressed relative to output of insulin recorded at 2.8 m*M* D-glucose or when the release of insulin provoked by L-leucine was compared in the presence vs absence of L-glutamine (Table 5, lower part). In such a case, the results obtained in FO-ω3 rats averaged 55.7 ± 3.6% ($n = 84$; $p < 0.001$) of those found in OO-ω3 rats (100.0 ± 8.4%; $n = 75$) Relative to the same reference value (OO-ω3 rats), the corresponding measurements made in normal rats yielded a mean value of 56.5 ± 2.8% ($n = 75$; $p < 0.001$) virtually identical ($p > 0.8$) to that found in the FO-ω3 rats. In this respect, the prior injection of the MCT:FO emulsion thus also restored a normal secretory behaviour of the isolated pancreatic islets.

Discussion

Only limited information is presently available on either the immediate and direct effects of ω3 on insulin secretion or the long-term modulation of pancreatic islet function by ω3. In a study conducted in β-TC3 insulinoma cells, C20:5ω3 was found to augment both glucose- and potassium-induced insulin secretion, without affecting glucose metabolism or intracellular free calcium *(14)*. Likewise, when tested at very high concentration (5 m*M*), α-linolenic acid was found to stimulate insulin release from perifused mouse islets, this effect being mimicked by 10 mmol/L C18:2ω6 *(15)*.

In a more physiological perspective, ob/ob mice fed a diet containing 23% fish oil were reported to display plasma insulin levels significantly higher 3–6 mo after the start of the diet than in animals fed diets containing either 25% lard or 25% safflower seed oil *(16)*. However, in normal male Wistar rats fed for 1 mo a diet containing 7% cod liver oil, instead of 7% corn oil, the strong hypolipidemic effect produced by the administration of fish oil was accompanied by a significant reduction of plasma insulin levels, both under basal conditions and after an intravenous glucose challenge, despite unchanged basal plasma glucose concentration and unchanged intravenous glucose tolerance *(17)*. Finally, in two closely related studies, Hollness et al. *(18,19)* reported that the substitution of a small amount (7%) of dietary lipid with ω3 during 4 wk of high-saturated fat feeding or during only the last 24 h of this 4 wk period reversed both insulin hypersecretion in vivo after an intravenous glucose challenge and the effect of long-term high-saturated fat feeding to enhance glucose-stimulated insulin secretion in isolated perifused islets. In the latter two studies, the administration of ω3 lowered the rates of glucose disappearance and increased the incremental integrated plasma glucose values during the intravenous glucose tolerance test, despite either a significant decrease or a modest unsignificant lowering of the insulin resistance index *(18,19)*.

The present study provides four major pieces of information.

First, the results obtained in vivo document a modest degree of insulin resistance in the ω3-depleted rats, these animals displaying a plasma glucose concentration in the high range of normal values, despite significantly increased plasma insulin concentration. The higher lipacidemia in the ω3-depleted rats is also consistent with such a proposal, the isolated adipocytes of these animals being indeed resistant to insulin in vitro *(7)*. The increased mass of adipose tissue in the ω3-depleted rats probably also contributes, however, to the increase in lipacidemia *(7)*. The lower concentration of triglycerides in the plasma of ω3-depleted rats, which confirms a recent observation made in older male ω3-depleted rats *(5)*, coincides, as expected, with a decrease in the plasma concentration of phospholipids. Our results suggest that both changes may be attributable to a decrease in the size rather than number of circulating lipid particles, e.g., VLDL. Indeed, and on the one hand, the values found in NI-ω3 rats, expressed relative to those measured in normal rats, yielded a much lower ratio ($p < 0.001$) in the case of triglycerides (39.9 ± 4.2%; d.f. = 10) than in the case of phospholipids (80.3 ± 3.8%; d.f. = 11), this ruling out a mere change in the number of lipid particles. On the other hand, and again relative to the values found in normal animals, the cube root of the plasma triglyceride concentration and the square root of the plasma phospholipid concentration averaged, in the NI-ω3 rats, respectively 73.6 ± 2.6% (d.f. = 10) and 89.9 ± 2.2% (d.f. = 11). The latter two percentages, which were both lower than unity ($p < 0.001$), remained significantly different from one another ($p < 0.001$). This finding is compatible with a preferential decrease in the size of large particles such as VLDL.

Second, the present results indicate that, as a rule, no significant difference is found between normal and ω3-depleted rats, as far as the protein, insulin, and lipid content of isolated islets is concerned. The trend was, however, toward a higher lipid content of the islets in ω3-depleted as compared to normal rats. Moreover, the fatty acid pattern of islet lipids was consistent with a prevailing concentration of phospholipid relative to triglycerides, as indicated by the high ponderal percentage of C20:4ω6. Moreover, the measurements made in both the pancreatic islets and plasma lipids clearly documented the deficiency in long-chain polyunsaturated ω3 fatty acids prevailing in the ω3-depleted rats. Finally, the data collected in leukocytes indicated the efficiency of the MCT:FO emulsion in provoking the enrichment of cell phospholipids in such ω3 fatty acids.

Third, two anomalies of D-glucose metabolism were observed in the islets from ω3-depleted rats. First, the relative magnitude of the increase in both D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation, in response to the progressive rise in D-glucose concentration was less pronounced in ω3-depleted rats than in normal animals. Because such a rise is primarily attributable to the participation of glucokinase in the phosphorylation of D-glucose, it seems obvious that the kinetics of the hexose phosphorylation are unfavourably affected in the ω3-depleted rats, as could, for instance, be the case if glucokinase would be inhibited, in the islets

of the latter rats, by an abnormally high concentration of long-chain fatty acid-coenzyme A. Second, the paired ratio between $^{14}CO_2$ production from D-[U-^{14}C]glucose and ^{3}HOH generation from D-[5-^{3}H]glucose was lower, at all concentrations of the hexose, in the islets from ω3-depleted rats than in those from normal animals. This impairment of the preferential stimulation of D-glucose oxidation relative to D-glucose utilization could also conceivably be related to a higher catabolism of endogenous fatty acids, such as those either generated by lipolysis of intracellular triglycerides or supplied to the islet cells from the bloodstream. In the latter respect, it was indeed recently demonstrated that the clearance of circulating non-esterified fatty acids is increased in ω3-depleted rats *(20)*. It should also be kept in mind that, in islets exposed to increasing concentrations of extracellular D-glucose, the hexose normally exerts a sparing action upon the oxidation of endogenous fatty acids, as documented in islets prelabelled with [U-^{14}C]palmitate *(21)*. There was little to distinguish between NI-ω3 and OO-ω3 rats, on the one hand, and FO-ω3 rats, on the other hand, in terms of the two anomalies of D-glucose metabolism in isolated islets. If anything, the trend was toward a correction of these defects in the FO-ω3 rats.

Finally, the secretory data reveal a higher rate of glucose-stimulated insulin release in ω3-depleted rats than in normal animals. This finding could conceivably be related to the insulin resistance of the former rats, because isolated islets, when explored in vitro, keep memory of long-term environmental regulatory factors, for instance in such situations as starvation or glucose intolerance *(22)*. A further difference between normal and ω3-depleted rats consisted in a higher responsiveness to either a rise in D-glucose concentration or non-glucidic nutrient secretagogues, relative to insulin secretion evoked by 8.3 m*M* D-glucose, in the NI-ω3 and/or OO-ω3 rats as compared, respectively, to the normal and/or FO-ω3 animals. In the light of the results concerning D-glucose metabolism in the islets, such a difference is unlikely to be mainly attributable to any major difference in the catabolic fate of the hexose in islet cells.

An alternative explanation could be related to the perturbation of cationic fluxes, especially K^+ and Ca^{2+} handling, in the islets of ω3-depleted rats, as documented in separate experiments *(9,11)*. Indeed, virtually the same anomaly as that documented in the present study in the case of nutrient-stimulated insulin release was observed in these other experiments, when considering the effects upon insulin release of non-nutrient secretagogues such as Ba^{2+}, high extracellular K^+ concentrations, theophylline, cytochalasin B, or the tumor-promoting agent phorbol 12-myristate 13-acetate *(10)*. Moreover, selected variables relative to K^+ and Ca^{2+} handling in the islets from ω3-depleted rats are also normalized after injection of the MCT:FO emulsion *(9,11)*.

In conclusion, the present study reveals a number of metabolic and hormonal perturbations in ω3-depleted rats. It also indicates that the prior injection of the MCT:FO emulsion corrects some but not all of these perturbations. Taken as a whole, therefore, our findings may help to understand the determinism of altered fuel homeostasis in situations characterized by a deficiency of long-chain polyunsaturated ω3 fatty acids.

Table 6
Fatty Acid Pattern (Weight Percentage) of Lipids in the Diets

Diet	Control	ω3-depleted
C16:0 (%)	13.4–13.8[a]	17.5–19.7
C16:1ω7 (%)	0.6–0.8	0.3–0.4
C18:0 (%)	2.3–2.6	9.8–11.3
C18:1ω9 (%)	16.7–17.7	42.1–45.0
C18:2ω6 (%)	54.1–54.8	12.8–20.7
C18:3ω6 (%)	N.D.[b]	0.2–0.3
C18:3ω3 (%)	5.9–6.4	0.2–0.3
C20:1ω9 (%)	0.9–0.9	0.4–0.5
C20:3ω6 (%)	0.0–0.1	0.6–0.6
C20:4ω6 (%)	0.2–0.2	0.3–0.3
C20:5ω3 (%)	0.6–0.8	0.2–0.6
C22:4ω6 (%)	0.2–0.2	0.4–0.5
C22:5ω3 (%)	0.1–0.2	0.2–0.2
C22:6ω3 (%)	1.4–1.6	0.6–0.7

[a]Lower and upper limit of separate determinations (n = 2–3).
[b]N.D.: not detected.

Materials and Methods

Diets and Emulsions

The control diet (AO3; SAFE, Villemoison-sur-Orge, France) contained (percent; w/w) 21 protein, 52 carbohydrate (including 34 starch), 5 lipid (soya oilcake), 4 cellulose, 5 of a vitamin and mineral mixture, and 12 water. The diet offered to the ω3-depleted rats contained (percent; w/w) 23 casein, 36 corn starch, 26 saccharose, 5 sunflower oil, 2 agar–agar, 2 cellulose, 5 of vitamin mixture and 1 of mineral mixture. The fatty acid pattern of the lipids in these two diets is documented in Table 6. The C18:3ω3 weight percentage was about 25 times lower in the ω3-depleted diet than in the control one. Other differences included higher C16:0, C18:0, and C18:1ω9 and lower C18:2ω6 relative contributions in the ω3-depleted diet than in the control one.

Table 7 provides the fatty acid pattern of the MCT:OO and MCT:FO emulsion. The most abundant fatty acids in the phospholipids were C16:0 (32.2 ± 0.1%), C18:1ω9 (24.4 ± 0.7%), C18:2ω6 (18.7 ± 0.6%), C18:0 (11.8 ± 0.5%), C20:4ω6 (6.1 ± 0.1%), and C22:6ω3 (3.8 ± 0.1%). Only minor differences in the weight percentage of C8:0, C10:0, and C20:5ω3 were found between the phospholipids of the two emulsions. The total fatty acid content of phospholipids averaged 8.9 ± 0.5 mg/mL.

The total fatty acid content of triglycerides was also comparable in the two emulsions, amounting to 174.1 ± 3.7 mg/mL. The most abundant fatty acids in the triglycerides were

Table 7
Fatty Acid Pattern of the MCT:OO and MCT:FO Emulsion

Emulsion	MCT:OO				MCT:FO			
Lipids[a]	PL	TG	DG	NEFA	PL	TG	DG	NEFA
Total (mg/mL)	9.4	177.8	1.3	0.7	8.4	170.4	12.4	1.1
C8:0 (%)	0.7	45.5	28.2	16.7	0.2	52.8	14.4	15.4
C10:0 (%)	0.3	34.1	30.8	12.4	0.1	34.8	9.6	11.6
C12:0 (%)	0.0	0.3	0.0	0.0	0.0	0.6	0.1	0.4
C14:0 (%)	0.3	0.0	0.0	6.1	0.2	0.1	0.4	3.8
C16:0 (%)	32.3	0.9	0.0	17.0	32.1	0.4	1.9	18.9
C16:1ω7 (%)	1.0	0.0	0.0	0.0	0.7	0.1	0.6	0.0
C18:0 (%)	11.3	0.7	0.0	8.0	12.3	0.4	2.3	8.0
C18:1ω9 (%)	25.1	15.5	32.8	31.1	23.7	0.9	5.6	21.8
C18:2ω6 (%)	18.1	2.6	8.0	8.4	19.2	0.1	0.7	7.5
C20:0 (%)	0.0	0.0	0.0	0.0	0.0	0.1	0.6	0.0
C18:3ω3 (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
C20:1ω9 (%)	0.2	0.0	0.0	0.0	0.1	0.0	0.4	0.0
C18:4ω3 (%)	0.0	0.0	0.0	0.0	0.0	0.2	1.1	0.0
C20:2ω6 (%)	0.2	0.0	0.0	0.0	0.3	0.0	0.0	0.0
C20:3ω6 (%)	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0
C22:0 (%)	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
C20:4ω6 (%)	6.0	0.0	0.0	0.0	6.2	0.2	2.2	2.2
C22:1ω9 (%)	0.0	0.0	0.0	0.0	0.0	0.1	0.7	0.0
C20:5ω3 (%)	0.0	0.0	0.0	0.0	0.3	4.0	26.3	4.3
C24:0 (%)	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
C22:4ω6 (%)	0.2	0.0	0.0	0.0	0.3	0.2	1.6	0.0
C22:5ω3 (%)	0.3	0.0	0.0	0.0	0.3	0.7	4.9	0.0
C22:6ω3 (%)	3.7	0.0	0.0	0.0	3.9	3.7	26.2	5.8

[a]PL: phospholipids; TG: triglycerides; DG: diglycerides; NEFA: non-esterified fatty acids.

C8:0 (49.2 ± 3.7%) and C10:0 (34.5 ± 0.4%). The long-chain polyunsaturated ω3 fatty acids C20:5ω3, C22:5ω3, and C22:6ω3 amounted to 8.4% in the triglycerides of the MCT:FO emulsion, while being undetectable in the triglycerides of the MCT:OO emulsion. Inversely, the weight percentage of C18:1ω9 (15.5 vs 0.9%) and C18:2ω6 (2.6 vs 0.1%) was much higher in the triglycerides of the MCT:OO as compared to MCT:FO emulsion.

The total fatty acid content of diglycerides was one order of magnitude higher in the MCT:FO emulsion (12.4 mg/mL) than in the MCT:OO emulsion (1.3 mg/mL). The long-chain polyunsaturated ω3 fatty acids C20:5ω3, C22:5ω3, and C22:6ω3 accounted for 57.4% of the total amount of fatty acids in the diglycerides of the MCT:FO emulsion, while being undetectable in the diglycerides of the MCT:OO emulsion. Once again, the weight percentage of C18:1ω9 (32.8 vs 5.6%) and C18:2ω6 (8.0 vs 0.7%) was much higher in the diglycerides of the MCT:OO as compared to MCT:FO emulsion. The weight percentage of the medium-chain fatty acids C8:0 and C10:0 was also higher in the diglycerides of MCT:OO emulsion (28.2 and 30.8%) as distinct from MCT:FO emulsion (14.4 and 9.6%).

The unesterified fatty acids only accounted for 0.5 ± 0.1% of the total amount of fatty acids present in the two emulsions, i.e., 190.7 ± 1.5 mg/mL. They consisted mainly of the medium-chain fatty acids C8:0 (16.1 ± 0.7%) and C10:0 (12.0 ± 0.4%), C16:0 (18.0 ± 1.0%), C18:1ω9 (31.1% in the MCT:OO emulsion and 21.8% in the MCT:FO emulsion), and C18:2ω6 (8.0 ± 0.5%). The long-chain polyunsaturated ω3 fatty acids C20:5ω3 and C22:5ω3 represented, respectively, 4.3 and 5.8% of the total amount of unesterified fatty acids in the MCT:FO emulsion, while being undetectable in the unesterified fatty acids of the MCT:OO emulsion.

Animals

Female normal rats (Iffa Credo, L'Arbresle, France) and ω3-depleted rats of comparable age (Table 1) were given free access to food and water throughout the study. The ω3-depleted rats were obtained as described elsewhere *(23)*. Briefly, 3-wk-old male and female rats (Elevage Janvier, Saint Genest, France) were made deficient using a sunflower-based diet as the only dietary lipid source. At 12 wk of age, females were mated with males and their resulting litters were culled to 10 pups. At weaning, they were maintained on the same diet as their parents. They were mated between males and females at 12 wk of age and the resulting pups were used for the study. These animals of second generation were maintained under the same dietary conditions until use.

Some ω3-depleted rats were injected in a tail vein with 1.0 mL of either the MCT:OO or MCT:FO emulsion 120 min before sacrifice. These animals are referred to as OO-ω3 and FO-ω3 rats. The ω3-depleted rats not injected with a lipid emulsion before sacrifice are indicated as NI-ω3.

The procedures used in this study were approved by the local Animal Experimentation Ethics Committee. The animals were sacrificed under CO_2 anesthesia.

Methods

The methods used to measure the plasma concentration of glucose *(24)*, insulin *(25)*, phospholipids, triglycerides, and unesterified fatty acids and to characterize the fatty acid pattern of circulating lipids *(26)* were previously described in the cited references. Likewise, the methods used for the isolation of circulating leukocytes *(27)* and pancreatic islets *(28)*, for the extraction, quantification, and analysis (fatty acid pattern) of tissue lipids *(26,29)*, for the determination of the protein *(30)*, insulin *(28)*, and glycerol *(31)* content of pancreatic islets, and for the measurement of the ^{3}HOH and L-[1-^{14}C]glucose distribution space *(32)*, the utilization of D-[5-^{3}H]glucose and oxidation of D-[U-^{14}C]glucose *(33)*, and the output of insulin *(28)* in incubated pancreatic islets were all already reported in prior publications.

Presentation of Results

All results are presented as the mean value (± 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 or, whenever so required, by variance analysis.

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