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Pancreatic islet function in ω -3 fatty acid-depleted rats: alteration of calcium fluxes and calcium-dependent insulin release

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long-chain polyunsaturated ω -3 fatty acid-depleted rats; calcium fluxes; pancreatic islets

BECAUSE OF THE PRESENTLY FREQUENT occurrence of a depletion in long-chain polyunsaturated ω -3 fatty acids in Western populations and its consequences in terms of the control of fuel homeostasis (1, 3, 12, 13), we have recently characterized several metabolic and hormonal variables in ω -3-depleted rats (second generation) and investigated their possible correction within 60–120 min after the bolus intravenous injection of a novel medium-chain triglyceride-fish oil (MCT:FO) emulsion found to provoke a rapid enrichment of cell phospholipids in C20:5 ω -3 and C22:6 ω -3 in both normal human subjects and

either normal or ω -3-depleted rats (10, 11). Selected metabolic, cationic, and secretory variables also were examined in isolated pancreatic islets (2, 6, 8) prepared from the ω -3-depleted rats and compared with those measured in islets from either normal rats or ω -3-depleted animals injected intravenously 120 min before death with the MCT:FO emulsion or a control ω -3 fatty acid-poor medium-chain triglyceride-olive oil (MCT:OO) emulsion. The present report deals with the study of Ca^{2+} fluxes and Ca^{2+} -dependent insulin secretory events in the isolated islets obtained from these different groups of rats.

MATERIALS AND METHODS

Female normal rats (Iffa Credo, L'Arbresle, France) and ω -3-depleted rats of comparable age (9a) were given free access to food and water up to the time of death. The ω -3-depleted rats (second generation) were obtained as described elsewhere (15). The procedures used in this study were approved by the Animal Experimentation Ethics Committee of Brussels Free University Medical School.

The control diet (AO3; SAFE, Villemoisson-sur-Orge, France) and that offered to the ω -3-depleted rats are described in detail elsewhere (9a). Briefly, the control and ω -3-depleted diet contained 5% (wt/wt) lipids from soya and sunflower, respectively, with the C18:3 ω -3 ponderal percentage in fatty acids being $\sim$ 25 times lower in the latter than in the former diet. Some ω -3-depleted rats were injected in a tail vein 120 min before death with 1.0 ml of either an ω -3 fatty acid-rich MCT:FO emulsion or a control MCT:OO emulsion (9a).

The methods used to measure the net uptake of $^{45}\text{Ca}^{2+}$ (5) and release of insulin (9) by isolated pancreatic islets and cytosolic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in islet cells (14) were previously described in the cited references. Briefly, for measuring $^{45}\text{Ca}^{2+}$ net uptake, groups of 10 islets each were incubated for 10 or 60 min in 0.1 ml of a bicarbonate- and HEPES-buffered medium containing bovine serum albumin (1.0 mg/ml), D-glucose (8.3 mM), and $^{45}\text{CaCl}_2$ (8.2 $\mu\text{Ci}/\text{ml}$) and placed below a layer (150 μl) of dibutyl phthalate. The islets were then separated from the incubation medium by centrifugation. For measuring insulin release, groups of eight islets each were incubated for 90 min in 1.0 ml of medium. For measuring $[\text{Ca}^{2+}]_i$, groups of 50,000 dispersed islet cells each obtained from either control or ω -3-depleted rats (3 experiments in each case) were cultured overnight on a round glass coverslip placed in petri dishes and then labeled with fura 2-acetoxymethyl ester (2 μM) over 60 min of incubation at 37°C. The coverslips were transferred to a tissue chamber mounted on an inverted fluorescence microscope (Diaphot TDM; Nikon, Tokyo, Japan) for epifluorescence. The cells were eventually perfused (1.4 ml/min) at 37°C using a Krebs-Ringer bicarbonate-buffered solution (pH 7.4) containing 137 mM NaCl, 5.4

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mM KCl, 1.2 mM CaCl_2 , 0.8 mM MgSO_4 , 0.3 mM Na_2HPO_4 , 0.4 mM KH_2PO_4 , 4.2 mM NaHCO_3 , and 8.3 mM D-glucose. To raise the K^+ concentration to 60 mM, we substituted NaCl with an equimolar amount of KCl. Fura 2 fluorescence of single cells was measured by dual-excitation fluorimetry using a camera-based image analysis system (Metafluor; Universal Imaging). The excitation and emission wavelengths were set at 340–380 and 510 nm, respectively, and a pair of rationable images was taken every 2.5 s.

All results are presented as means $\pm$ SE together with either the number of separate determinations (n) or degrees of freedom (df). The statistical significance of differences between mean values was assessed using Student's t -test.

RESULTS

$^{45}\text{Ca}^{2+}$ data. The mean absolute values for $^{45}\text{Ca}^{2+}$ net uptake by islets prepared from normal rats and ω -3-depleted rats injected 2 h before death with either the MCT:OO or MCT:FO emulsion and incubated for 10 and 60 min at increasing concentrations of extracellular K^+ (5, 30, and 60 mM) are given in Table 1. In all cases, the $^{45}\text{Ca}^{2+}$ uptake was much higher ($P < 0.001$) after 60 min of incubation than after only 10 min of incubation. The values recorded in islets prepared from the same type of rats and incubated at increasing concentrations of extracellular K^+ also are different from one another ($P < 0.02$ or less), except when the islets from ω -3-depleted rats injected with the MCT:FO emulsion and incubated for 10 or 60 min at 5 vs. 30 mM K^+ are compared ($P > 0.2$ or more).

Judging from the net uptake of $^{45}\text{Ca}^{2+}$ after 10 min of incubation, the inflow rate of the divalent cation was significantly lower ($P < 0.025$ or less) in ω -3-depleted rats injected with the MCT:OO emulsion than in normal rats when the islets were incubated at 5 or 30 mM K^+ . Such a difference faded out ($P > 0.4$), however, at 60 mM K^+ . In this respect, there was no significant difference between ω -3-depleted rats injected with

the MCT:OO emulsion or those injected with the MCT:FO emulsion, except for the lower value ($P < 0.02$) found in the latter than in the former rats in islets exposed to 30 mM K^+ .

To more precisely assess the effect of increasing concentrations of extracellular K^+ (5, 30, and 60 mM) on $^{45}\text{Ca}^{2+}$ net uptake, we expressed the results recorded after 10 or 60 min of incubation at the two lowest K^+ concentrations (5 and 30 mM) relative to the mean corresponding values found at the same incubation time and within the same experiment(s) in islets exposed to 60 mM K^+ . With the results found after 10 and 60 min of incubation pooled together, the net uptake of $^{45}\text{Ca}^{2+}$ averaged $46.2 \pm 2.3\%$ ($n = 54$) and $67.9 \pm 2.5\%$ ($n = 51$) at 5 and 30 mM K^+ , respectively, relative to the value found at 60 mM K^+ , in normal rats and $45.9 \pm 3.3\%$ ($n = 42$) and $65.2 \pm 3.4\%$ ($n = 46$) in ω -3-depleted rats injected with the MCT:OO emulsion, with there being no significant difference ($P > 0.5$ or more) between these two types of animals. In the ω -3-depleted rats injected with the MCT:FO emulsion, the value found at 5.0 mM K^+ ($45.0 \pm 4.0\%$; $n = 44$) was virtually identical ($P > 0.8$) to that found in the ω -3-depleted rats injected with the MCT:OO emulsion. At 30.0 mM K^+ , however, the value found in the ω -3-depleted rats injected with the MCT:FO emulsion ($53.1 \pm 4.1\%$; $n = 43$) was significantly lower ($P < 0.03$ or less) than that found in either normal animals or the ω -3-depleted rats injected with the MCT:OO emulsion.

The time course for $^{45}\text{Ca}^{2+}$ net uptake also differed in the normal vs. ω -3-depleted rats. Thus, relative to the mean value found within the same experiment(s) at the same K^+ concentration after 60 min of incubation, the results found after only 10 min of incubation averaged $52.0 \pm 1.9\%$ ($n = 78$) in normal rats, distinct ($P < 0.001$) from $32.0 \pm 2.4\%$ ($n = 68$) and $33.9 \pm 2.7\%$ ($n = 63$) in the ω -3-depleted rats injected with the MCT:OO and MCT:FO emulsion, respectively.

Table 1. $^{45}\text{Ca}^{2+}$ uptake and fluxes in isolated pancreatic islets

	K^+ , mM		
	5.0	30.0	60.0
<i>Normal rats</i>			
$^{45}\text{Ca}^{2+}$ uptake, pmol/islet			
10 min	3.47 ± 0.20 (27)	5.23 ± 0.28 (24)	7.01 ± 0.39 (27)
60 min	6.54 ± 0.47 (27)	9.68 ± 0.72 (27)	16.36 ± 1.48 (26)
$^{45}\text{Ca}^{2+}$ fractional outflow rate, %/min	7.43 ± 1.02	7.65 ± 1.08	5.29 ± 0.73
$^{45}\text{Ca}^{2+}$ pool, pmol/islet	6.62 ± 0.62	9.78 ± 0.92	17.08 ± 1.82
$^{45}\text{Ca}^{2+}$ inflow-outflow, fmol $\cdot$ min $^{-1}$ $\cdot$ islet $^{-1}$	492 ± 57	748 ± 91	903 ± 110
<i>ω-3-Depleted MCT:OO rats</i>			
$^{45}\text{Ca}^{2+}$ uptake, pmol/islet			
10 min	2.64 ± 0.31 (22)	3.84 ± 0.38 (23)	6.44 ± 0.61 (23)
60 min	10.25 ± 1.09 (20)	14.41 ± 1.29 (23)	19.71 ± 1.59 (22)
$^{45}\text{Ca}^{2+}$ fractional outflow rate, %/min	1.96 ± 0.34	2.14 ± 0.31	3.31 ± 0.49
$^{45}\text{Ca}^{2+}$ pool, pmol/islet	14.81 ± 2.43	19.92 ± 2.67	22.84 ± 2.90
$^{45}\text{Ca}^{2+}$ inflow-outflow, fmol $\cdot$ min $^{-1}$ $\cdot$ islet $^{-1}$	290 ± 49	426 ± 59	756 ± 104
<i>ω-3-Depleted MCT:FO rats</i>			
$^{45}\text{Ca}^{2+}$ uptake, pmol/islet			
10 min	2.35 ± 0.30 (20)	2.59 ± 0.33 (21)	6.22 ± 1.02 (22)
60 min	8.01 ± 0.85 (24)	9.56 ± 0.93 (22)	17.67 ± 1.61 (22)
$^{45}\text{Ca}^{2+}$ fractional outflow rate, %/min	2.67 ± 0.52	2.23 ± 0.40	3.80 ± 0.87
$^{45}\text{Ca}^{2+}$ pool, pmol/islet	10.03 ± 1.78	12.96 ± 1.76	19.69 ± 3.81
$^{45}\text{Ca}^{2+}$ inflow-outflow, fmol $\cdot$ min $^{-1}$ $\cdot$ islet $^{-1}$	268 ± 50	289 ± 46	748 ± 157

Values are means $\pm$ SE; nos. in parentheses indicate the no. of separate determinations for each value. ω -3-Depleted rats were injected with either medium-chain triglyceride-olive oil (MCT:OO) or MCT-fish oil (MCT:FO) emulsion.

Advantage was then taken of the measurements made after 10 and 60 min of incubation to estimate both the fractional turnover rate (k , expressed as %/min) and net uptake of $^{45}\text{Ca}^{2+}$ at isotopic equilibrium ($U_{\max}$, expressed as pmol/islet) according to the equation $U = U_{\max} (1 - e^{-kt})$, in which U represents the uptake of $^{45}\text{Ca}^{2+}$ (pmol/islet) at time t (expressed in minutes). As shown in Table 1, the $^{45}\text{Ca}^{2+}$ fractional turnover rate was lower in ω -3-depleted rats than in normal animals. At 5 and 30 mM K^+ , in the former rats it averaged $39.0 \pm 4.2\%$ ($n = 4$; $P < 0.001$) of the corresponding value found at the same K^+ concentration in normal animals. Such a percentage was increased ($P < 0.02$) to $65.3 \pm 2.5\%$ ($n = 2$) at 60 mM K^+ but remained lower ($P < 0.05$) than that found at the same high K^+ concentration in normal animals.

At isotopic equilibrium, the estimated $^{45}\text{Ca}^{2+}$ net uptake ($U_{\max}$) was highest in the ω -3-depleted rats injected with the MCT:OO emulsion and lowest in normal animals. As shown in Fig. 1, when the results found in the ω -3-depleted rats were expressed relative to those found at the same K^+ concentration in normal rats, the following findings became evident. First, in relative terms, the difference between ω -3-depleted rats and normal animals progressively decreased as the K^+ concentration was raised from 5 to 30 and 60 mM. Second, the results found in the ω -3-depleted rats injected with the MCT:OO and MCT:FO emulsion differed vastly from one another. Thus the slope of the regression line relating the normalized values for $U_{\max}$ (i.e., those expressed relative to the value found at the same K^+ concentration in normal rats) to the extracellular K^+ concentration was almost five times steeper ($P < 0.05$) in the ω -3-depleted rats injected with the MCT:OO emulsion (0.0135) than in the ω -3-depleted rats injected with the MCT:FO emulsion (0.0029). Moreover, in the range of K^+ concentrations under consideration, and as judged by covariance analysis, the elevation of the regression line also was significantly higher ($P < 0.05$) in the ω -3-depleted rats injected with the MCT:OO compared with the MCT:FO emulsion.

Last, and as expected, the estimation of $^{45}\text{Ca}^{2+}$ inflow-outflow rate at isotopic equilibrium provided, in essence, the same information as the net uptake of $^{45}\text{Ca}^{2+}$ after only 10 min of incubation (see above), with a correlation coefficient between the two sets of data amounting to 0.9774 ($df = 7$; $P < 0.001$). Also, as expected from the exponential time course for $^{45}\text{Ca}^{2+}$ net uptake, the experimental values measured after 10 min of incubation, when converted to pmol/min per islet, were somewhat lower ($P < 0.001$) than the estimated value for $^{45}\text{Ca}^{2+}$ inflow-outflow rate at isotopic equilibrium, with a paired ratio between these two variables of $82.8 \pm 2.7\%$ ($n = 9$).

Incidentally, the calculated values for $^{45}\text{Ca}^{2+}$ fractional outflow rate in the present study were sizably higher than those otherwise found during perfusion of prelabeled islets (4). This difference can be accounted for by the fact that the present data were not corrected for extracellular contamination, whereas the measurements of $^{45}\text{Ca}^{2+}$ outflow from prelabeled perfused islets are usually measured from the 31st minute of perfusion onward, namely, after washout of $^{45}\text{Ca}^{2+}$ from both the extracellular space and the most rapidly exchangeable cellular pool(s).

Secretory data. Several sets of experiments were conducted to compare the secretory behavior of islets obtained from either normal rats or ω -3-depleted animals, with the latter rats being

Fig. 1. Estimated $^{45}\text{Ca}^{2+}$ pool at isotopic equilibrium in islets prepared from normal rats (open bars; ○) and ω -3-depleted animals injected with either the medium-chain triglyceride-olive oil (MCT:OO) (horizontally hatched bars; ▲) or medium-chain triglyceride-fish oil (MCT:FO) emulsion (vertically hatched bars; ●) and incubated at increasing concentrations of extracellular K^+ is expressed either in absolute terms (top) or relative to results found at the same K^+ concentration in islets from normal rats (bottom). The 3 lines drawn at bottom were calculated by regression analysis.

injected (or not) 120 min before death with a lipid emulsion. The release of insulin provoked by 8.3 mM D-glucose at normal extracellular Ca^{2+} concentration (1.0 mM), which was measured in each experiment, was comparable in islets obtained from ω -3-depleted rats whether injected or not with a lipid emulsion, with an overall mean value of $137.8 \pm 8.9 \mu\text{U}/90$ min per islet ($n = 141$) higher ($P < 0.005$) than that found in normal animals ($91.6 \pm 4.2 \mu\text{U}/90$ min per islet; $n = 58$).

At 8.3 mM D-glucose and relative to the basal value recorded within the same experiment(s) in the presence of only 2.8 mM D-glucose, the output of insulin was higher in the MCT:OO-injected rats than in the MCT:FO-injected rats (Fig. 2). Indeed, in the former rats it averaged $447.2 \pm 104.1\%$ ($n = 13$; $P < 0.005$ vs. a basal value of $100.0 \pm 25.5\%$, $n = 16$), distinct ($P < 0.03$) from only $281.7 \pm 43.2\%$ ($n = 17$; $P < 0.001$ vs.

Fig. 2. Comparison between the release of insulin by islets obtained from ω -3-depleted rats injected 120 min before death with either MCT:OO (open bars) or MCT:FO emulsion (hatched bars). Each pair of bars refers to the reference value taken as unity (left) and the corresponding experimental value (right) as obtained under the conditions indicated for both the reference (top) and experimental (bottom) insulin output (2.8 or 8.3 G, 2.8 or 8.3 mM D-glucose; no or 1.0 Ca, no or 1.0 mM Ca^{2+} ; V, 20 μM verapamil). Data are means $\pm$ SE derived from 10–20 separate determinations.

a basal value of $100.0 \pm 8.5\%$, $n = 15$) in the MCT:FO-injected animals. Likewise, in the MCT:OO-injected rats, the output of insulin evoked by 8.3 mM D-glucose at normal extracellular concentration, relative to that recorded within the same experiment(s) at the same hexose concentration but in the nominal absence of Ca^{2+} , averaged $252.7 \pm 20.0\%$ ($n = 14$; $P < 0.001$ vs. a reference value of $100.0 \pm 11.8\%$, $n = 19$), distinct ($P < 0.005$) from $179.8 \pm 11.2\%$ ($n = 20$; $P < 0.001$ vs. a reference value of $100.0 \pm 12.7\%$, $n = 15$) in the MCT:FO-injected rats.

These findings also indicate that in both MCT:OO- and MCT:FO-injected rats, the release of insulin recorded in the presence of 2.8 mM D-glucose and 1.0 mM Ca^{2+} was lower than that found in the presence of 8.3 mM D-glucose but the absence of Ca^{2+} . Relative to the secretory rate found within the same experiment(s) at 8.3 mM D-glucose and normal extracellular concentration, that recorded in the presence of 2.8 mM D-glucose and 1.0 mM Ca^{2+} only represented $69.9 \pm 9.0\%$ ($n = 31$; $P < 0.025$) of that found in the same type of rats (MCT:OO- or MCT:FO-injected animals) at 8.3 mM D-glucose but in the absence of Ca^{2+} ($100.0 \pm 9.1\%$; $n = 34$). Such a situation differs from that found in the present study in islets

from normal rats, in which the release of insulin evoked by either 2.8 mM D-glucose at 1.0 mM Ca^{2+} or 8.3 mM D-glucose in the nominal absence of Ca^{2+} , relative to that found within the same experiment(s) in the presence of 8.3 mM D-glucose and 1.0 mM Ca^{2+} , was not significantly different, averaging 26.2 ± 2.3 and $18.5 \pm 2.9\%$, respectively ($n = 16$ –20).

In a further series of experiments, the release of insulin evoked by 8.3 mM D-glucose relative to that recorded at the same hexose concentration in the presence of the organic Ca^{2+} antagonist verapamil (20 μM) was again significantly higher ($P < 0.001$) in the MCT:OO-injected rats ($923.0 \pm 50.9\%$ vs. $100.0 \pm 16.8\%$; $n = 10$ –11; $P < 0.001$) than in the MCT:FO-injected animals ($426.0 \pm 34.6\%$ vs. $100.0 \pm 23.4\%$; $n = 12$ –15; $P < 0.001$). In other words, the low output for insulin recorded at 2.8 mM D-glucose and 1.0 mM Ca^{2+} , at 8.3 mM D-glucose in the absence of Ca^{2+} , and at 8.3 mM D-glucose in the presence of verapamil, compared with that evoked by 8.3 mM D-glucose at 1.0 mM Ca^{2+} , was always higher in MCT:FO-injected rats than in MCT:OO-injected animals. In this further series of experiments, the results obtained in the islets from ω -3-depleted rats injected with the MCT:OO emulsion, in terms of the ratio in insulin output at 8.3 mM D-glucose and 1.0 mM Ca^{2+} in the absence vs. presence of verapamil, were not significantly different ($df = 27$; $P > 0.3$) from those recorded in islets from ω -3-depleted rats not injected with any emulsion, with the latter results yielding a ratio 2.35 ± 0.24 times higher ($df = 37$; $P < 0.001$) than that recorded in islets from normal rats. This multiplication factor was similar ($P > 0.5$) to that calculated by comparison of the data obtained under the same experimental conditions in ω -3-depleted rats injected with the MCT:OO vs. MCT:FO emulsion (2.17 ± 0.14 ; $df = 23$; $P < 0.001$).

In the experiments illustrated in Fig. 2, the normalized values for insulin output recorded in the presence of 8.3 mM D-glucose at normal Ca^{2+} concentration (1.0 mM) in the ω -3-depleted rats injected with the MCT:FO emulsion averaged $61.3 \pm 3.9\%$ ($n = 52$; $P < 0.001$) of the mean corresponding values found in the ω -3-depleted rats injected with the MCT:OO emulsion ($100.0 \pm 8.6\%$; $n = 37$).

The second series of experiments concerned mainly the effect of various environmental cationic factors on insulin secretion (Fig. 3). A first difference between ω -3-depleted rats injected with the MCT:FO vs. the MCT:OO emulsion is the fact that the release of insulin recorded in the presence of D-glucose but the absence of Ca^{2+} , compared with that found within the same experiment(s) in the absence of both the hexose and divalent cation, averaged $167.5 \pm 21.7\%$ ($n = 19$) in MCT:OO-injected rats, distinct ($P < 0.001$) from $70.9 \pm 8.5\%$ ($n = 15$) in MCT:FO-injected animals. These two percentages are significantly different ($P < 0.02$ in both cases) from the corresponding mean reference values ($100.0 \pm 16.5\%$; $n = 19$ and $100.0 \pm 6.5\%$; $n = 15$). The value found in the islets from ω -3-depleted rats injected with the MCT:FO emulsion was virtually identical ($P > 0.975$) to that found under the same experimental conditions in islets obtained from normal rats, i.e., $70.3 \pm 16.3\%$ ($n = 20$).

Likewise, at normal extracellular Ca^{2+} concentration (1.0 mM), the release of insulin evoked by 8.3 mM D-glucose in the presence of theophylline (1.4 mM), relative to that found within the same experiment(s) in the absence of the phosphodiesterase inhibitor, was higher ($P < 0.05$) in the ω -3-depleted

Fig. 3. Comparison between the release of insulin by islets obtained from ω -3-depleted rats injected 120 min before death with either MCT:OO (open bars) or MCT:FO emulsion (hatched bars). Each pair of bars refers to the reference value taken as unity (*left*) and the corresponding experimental value (*right*) as obtained under the conditions indicated for both the reference (*top*) and experimental (*bottom*) insulin output (Ba, 1.0 mM Ba^{2+} ; K, 30 mM K^{+} ; P, 1.0 μM phorbol 12-myristate 13-acetate; T, 1.4 mM theophylline). Data are means $\pm$ SE derived from 10–20 separate determinations.

rats injected with the MCT:OO emulsion ($358.6 \pm 33.8\%$ vs. a reference value of $100.0 \pm 28.8\%$; $n = 14$ – 19 ; $P < 0.001$) than in the ω -3-depleted rats injected with the MCT:FO emulsion ($249.5 \pm 33.7\%$ vs. a reference value of $100.0 \pm 9.9\%$; $n = 17$ – 19 ; $P < 0.001$). In the islets from the latter rats, the theophylline-induced increment in insulin output ($+149.5 \pm 36.9\%$; $\text{df} = 34$) was not significantly different ($P > 0.2$) from that found in islets from normal rats ($+101.9 \pm 17.1\%$; $\text{df} = 37$; $P < 0.001$).

In the presence of 8.3 mM D-glucose, a rise in extracellular Ca^{2+} concentration from 1.0 to 4.0 mM again augmented insulin output to a greater relative extent ($P < 0.05$) in ω -3-depleted rats injected with the MCT:OO emulsion ($+53.1 \pm 13.9\%$; $\text{df} = 29$) than in ω -3-depleted rats injected with the MCT:FO emulsion ($+13.9 \pm 13.4\%$; $\text{df} = 36$). As a matter of fact, such an increase was highly significant ($P < 0.001$) in the former animals while failing to achieve statistical significance ($P > 0.3$) in the latter rats. Once again, the release of insulin recorded in the presence of 4.0 mM Ca^{2+} , relative to that found within the same experiment(s) at normal extracellular Ca^{2+} concentration, was not significantly different ($P > 0.5$) in the ω -3-depleted rats injected with the MCT:FO emulsion ($113.9 \pm 12.4\%$; $n = 18$) and in normal rats ($123.1 \pm 10.1\%$; $n = 20$), whereas the latter value remained lower ($P < 0.06$) than that found in ω -3-depleted rats injected with the MCT:OO emulsion ($153.1 \pm 12.3\%$; $n = 17$).

In a further set of experiments, the relative magnitude of the enhancing action of the tumor-promoting agent phorbol 12-myristate 13-acetate (PMA; 1.0 μM) on insulin release evoked by 8.3 mM D-glucose was once again found to be higher ($P < 0.02$) in MCT:OO-injected ω -3-depleted rats ($186.7 \pm 8.7\%$ vs. $100.0 \pm 5.5\%$; $n = 10$ – 12 ; $P < 0.001$) than in MCT:FO-

injected ω -3-depleted animals ($147.2 \pm 11.0\%$ vs. $100.0 \pm 8.1\%$; $n = 15$ in both cases; $P < 0.005$). Likewise, relative to the output of insulin recorded in the sole presence of 8.3 mM D-glucose, that recorded in the concomitant presence of PMA was 1.36 $\pm$ 0.10 times higher ($\text{df} = 38$; $P < 0.005$) in islets from ω -3-depleted rats not injected with any emulsion than in islets obtained from normal rats. This multiplication factor was comparable ($P > 0.5$) to that found by comparison of the data obtained under the same experimental conditions in ω -3-depleted rats injected with the MCT:OO emulsion vs. MCT:FO emulsion (1.26 ± 0.10 ; $\text{df} = 25$; $P < 0.02$).

A similar situation prevailed when comparing, in the presence vs. absence of Ba^{2+} (1.0 mM), the release of insulin from islets deprived of exogenous nutrient and incubated in the nominal absence of extracellular Ca^{2+} . In the islets from ω -3-depleted rats injected with the MCT:OO emulsion, the results recorded in the presence of Ba^{2+} averaged $217.1 \pm 43.6\%$ ($n = 17$; $P < 0.02$) of the mean corresponding values found within the same experiment(s) in its absence ($100.0 \pm 14.5\%$; $n = 19$). In the ω -3-depleted rats injected with the MCT:FO emulsion, however, the release of insulin recorded in the presence of Ba^{2+} averaged no more than $87.8 \pm 8.9\%$ ($n = 18$; $P > 0.3$) of that found in its absence ($100.0 \pm 9.2\%$; $n = 15$). The former percentage was significantly lower ($P < 0.01$) than that found in the ω -3-depleted rats injected with the MCT:OO emulsion and was not significantly different from that found in normal rats (i.e., $120.5 \pm 20.5\%$; $n = 17$; $P > 0.4$ vs. the reference value of $100.0 \pm 13.3\%$; $n = 15$).

A further difference between MCT:OO- and MCT:FO-injected rats observed in these experiments concerns the effect of theophylline (1.4 mM) on the output of insulin recorded in the islets deprived of both exogenous nutrient and extracellular

Ca^{2+} but exposed to Ba^{2+} (1.0 mM). In absolute terms, theophylline augmented insulin output by $12.7 \pm 5.6 \mu\text{U}/\text{islet}$ per 90 min ($\text{df} = 27$; $P < 0.04$) in the MCT:OO-injected rats and by $9.2 \pm 4.1 \mu\text{U}/\text{islet}$ per 90 min ($\text{df} = 31$; $P < 0.04$) in the MCT:FO-injected animals, with these two increments not significantly different from one another. However, in relative terms, such an increment was much lower ($P < 0.02$) in the MCT:FO-injected rats ($+26.6 \pm 13.4\%$; $\text{df} = 33$) than in the MCT:OO-injected rats ($+116.5 \pm 35.7\%$; $\text{df} = 29$), as already observed when considering the effect of theophylline on insulin secretion provoked by 8.3 mM D-glucose (see above).

Last, when the concentration of extracellular K^+ was raised from 5 to 30 mM, by isomolar partial substitution of NaCl by KCl, the release of insulin evoked by 8.3 mM D-glucose was, unexpectedly, not significantly affected ($P > 0.975$) in the islets from MCT:OO-injected rats and, paradoxically, decreased to $82.0 \pm 5.5\%$ ($n = 14$; $P < 0.05$) of the mean corresponding value found within the same experiment(s) at 5 mM K^+ ($100.0 \pm 6.1\%$; $n = 20$) in the islets from MCT:FO-injected animals (Fig. 3).

In the last two series of experiments dealing with the effects of theophylline on Ba^{2+} -induced insulin release and of the rise in extracellular K^+ concentration on glucose-induced insulin secretion, the situation found in islets from ω -3-depleted rats injected with the MCT:FO emulsion remained different from that found in islets isolated from normal rats. Thus, on one hand, in the islets from normal rats, theophylline augmented the output of insulin caused by Ba^{2+} in the absence of Ca^{2+} to $299.3 \pm 38.7\%$ ($n = 19$; $P < 0.001$) of the mean value found in the absence of the phosphodiesterase inhibitor ($100.0 \pm 16.2\%$; $n = 17$). On the other hand, in the islets from normal rats, the rise in K^+ concentration to 30 mM increased insulin output provoked by D-glucose (8.3 mM) to $129.8 \pm 14.2\%$ ($n = 17$) of the mean corresponding value found within the same experiments at 5.0 mM K^+ ($100.0 \pm 9.5\%$; $n = 19$). However, this increase failed to achieve statistical significance ($P > 0.07$).

In the experiments illustrated in Fig. 3, the normalized values for insulin output, as expressed relative to the selected reference value, in the ω -3-depleted rats injected with the MCT:FO emulsion averaged $63.3 \pm 2.8\%$ ($n = 116$; $P < 0.001$) of the mean corresponding values found in the ω -3-depleted rats injected with the MCT:OO emulsion ($100.0 \pm 4.5\%$; $n = 120$). The former percentage was virtually identical ($P > 0.6$) to that found in the experiments illustrated in Fig. 2, i.e., $61.3 \pm 3.9\%$ ($n = 52$).

Cytosolic Ca^{2+} concentration. Figure 4 shows the time course for changes in $[\text{Ca}^{2+}]_i$ measured in islet cells obtained from either normal rats or ω -3-depleted animals (not injected with any emulsion) and examined at 8.3 mM D-glucose, first at normal (5.0 mM) and then at high (60.0 mM) extracellular K^+ concentration. At time 0 and 10 min, respectively, the $[\text{Ca}^{2+}]_i$ was $34 \pm 11 \text{ nM}$ ($P < 0.005$; $\text{df} = 311$) and $31 \pm 12 \text{ nM}$ ($P < 0.01$; $\text{df} = 297$) higher in the cells from ω -3-depleted rats than in those from normal rats. These two values were not significantly different ($P > 0.8$) from one another. They are consistent with the above-mentioned finding that at 8.3 mM D-glucose, the secretion of insulin by islets from ω -3-depleted rats exceeds that found in islets from normal animals.

The rise in K^+ concentration provoked a rapid increase in $[\text{Ca}^{2+}]_i$, peaking within 3 min at mean values of $414 \pm 29 \text{ nM}$

Fig. 4. Time course for changes in cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in dispersed islet cells from uninjected ω -3-depleted rats (top) and control animals (bottom). The cells were exposed to 8.3 mM D-glucose throughout, with the extracellular K^+ concentration raised from 5.0 to 60.0 mM at 10 min. Data are means $\pm$ SE derived from 179 (ω -3-depleted) and 134 (control) individual cells at time 0.

($n = 175$) and $373 \pm 36 \text{ nM}$ ($n = 118$) in cells from ω -3-depleted and normal rats, respectively. This coincided in most cells with induction of $[\text{Ca}^{2+}]_i$ oscillations. The mean $[\text{Ca}^{2+}]_i$ then progressively decreased. The time course for these changes in $[\text{Ca}^{2+}]_i$ was quite similar to the known pattern of insulin release provoked in isolated islets by a rise in extracellular K^+ concentration (5).

After 15 min of exposure to 60 mM K^+ , the $[\text{Ca}^{2+}]_i$ was $58 \pm 16 \text{ nM}$ ($P < 0.001$; $\text{df} = 271$) higher in the islet cells from ω -3-depleted rats than in those from normal animals. The latter difference was about two times higher than that recorded before the extracellular K^+ concentration was raised. As a matter of fact, after 15 min of exposure to 60 mM K^+ , the $[\text{Ca}^{2+}]_i$ remained significantly higher ($P < 0.01$) than that recorded at time 0 in the islet cells from ω -3-depleted rats, whereas such was not the case ($P > 0.5$) in the islet cells from normal animals. Moreover, covariance analysis indicates that during the period covered by the last 100 measurements, the mean $[\text{Ca}^{2+}]_i$ decreased at a rate of $26.0 \pm 1.2 \text{ nM}/\text{min}$ in the islet cells from normal rats, distinct ($P < 0.001$) from only $4.0 \pm 0.6 \text{ nM}/\text{min}$ in the cells from ω -3-depleted animals. These findings strongly suggest that over 90 min of exposure to an elevated K^+ concentration, the mean value for $[\text{Ca}^{2+}]_i$, expressed relative to that recorded at normal K^+ concentration (5.0 mM), would be significantly higher in the islet cells from ω -3-depleted rats than in those from normal animals, once again in agreement with the corresponding secretory data (Fig. 3).

DISCUSSION

The present study affords novel information on three inter-related items, namely, the handling of $^{45}\text{Ca}^{2+}$ and Ca^{2+} dependency of insulin release in islets from ω -3-depleted rats and the cytosolic Ca^{2+} concentration in dispersed islet cells from the same animals. Concerning the first of these three issues, our results indicate that with the sole possible exception of islets prepared from ω -3-depleted rats injected with the MCT:FO emulsion and exposed to 30 mM K^+ , the response to an increase in extracellular K^+ concentration, in terms of stimu-

lation of $^{45}\text{Ca}^{2+}$ influx, was essentially comparable in ω -3-depleted rats and normal rats. For instance, after 10 min of incubation, the net uptake of $^{45}\text{Ca}^{2+}$ at 5 mM K^+ , relative to that found within the same experiment(s) at 60 mM K^+ , averaged $49.5 \pm 2.9\%$ ($n = 27$) in normal rats, $40.2 \pm 4.4\%$ ($n = 22$) in ω -3-depleted rats injected with the MCT:OO emulsion, and $45.6 \pm 7.2\%$ ($n = 20$) in ω -3-depleted rats injected with the MCT:FO emulsion, with none of these values being significantly different from one another. The apparently unchanged responsiveness to plasma membrane depolarization of voltage-sensitive Ca^{2+} channels in the ω -3-depleted rats was supported by the finding that after 10 min of incubation at 60 mM K^+ , the net uptake of $^{45}\text{Ca}^{2+}$ was not significantly different ($P > 0.4$) in normal rats (7.01 ± 0.39 pmol/islet; $n = 27$) and ω -3-depleted rats (6.33 ± 0.58 pmol/islet; $n = 45$). Nevertheless, at 5 or 30 mM K^+ , the influx of Ca^{2+} into the islet cells, as judged from the net uptake of $^{45}\text{Ca}^{2+}$ after 10 min of incubation, was significantly lower in ω -3-depleted rats than in normal animals. For instance, at 5 mM K^+ , it averaged 2.50 ± 0.21 pmol/islet in ω -3-depleted rats, distinct ($P < 0.005$) from 3.47 ± 0.20 pmol/islet in normal animals. Such a difference cannot be blamed on a lower size of the islets from ω -3-depleted rats compared with those from normal rats. On the contrary, the protein content of the islets is somewhat higher, although not significantly so ($P > 0.1$) in ω -3-depleted rats (1.38 ± 0.15 μg /islet; $n = 36$) than in control animals (0.98 ± 0.08 μg /islet; $n = 14$). Because the present experiments were conducted in the presence of 8.3 mM D-glucose, the low influx of Ca^{2+} found at 5 mM K^+ in the islets from ω -3-depleted rats, distinct from that in normal animals, could conceivably be attributed, at least in part, to the impairment of D-glucose metabolism recently documented in the islets from ω -3-depleted rats and characterized by both a lesser increase of D-glucose utilization and oxidation in response to a rise in hexose concentration and a low ratio between D-glucose oxidation and utilization (2). This first anomaly of Ca^{2+} fluxes in the islets of ω -3-depleted rats was not corrected by the prior intravenous administration of the MCT:FO emulsion, distinct from the MCT:OO emulsion, to such animals. This coincides with the fact that the same procedure also fails to correct the impairment of D-glucose metabolism in the islets from ω -3-depleted rats.

A second anomaly of $^{45}\text{Ca}^{2+}$ handling in the islets from ω -3-depleted rats is the lower fractional outflow rate of the divalent cation ($P < 0.001$) ($2.69 \pm 0.30 \cdot 10^{-2} \cdot \text{min}^{-1}$; $n = 6$) compared with that in normal rats ($6.79 \pm 0.75 \cdot 10^{-2} \cdot \text{min}^{-1}$; $n = 3$). Because the efflux of Ca^{2+} from the islet cells is mediated by both a plasma membrane-associated Ca^{2+} -ATPase and $\text{Na}^+/\text{Ca}^{2+}$ countertransport, this finding suggests alteration of one or both of these two processes in the ω -3-depleted rats. This second perturbation of Ca^{2+} fluxes in the islets from ω -3-depleted rats apparently also failed to be corrected by the injection of the MCT:FO emulsion 120 min before death.

The third major perturbation of $^{45}\text{Ca}^{2+}$ handling by the islets from ω -3-depleted rats consisted of higher values for the estimated size of the $^{45}\text{Ca}^{2+}$ -labeled cellular pool(s) at postulated isotopic equilibrium (Fig. 1). In considering this last finding, it should be stressed that such an estimation only concerns those cellular pools of the divalent cation with a relatively high fractional turnover rate, because the present

measurements were restricted to the first 60 min of incubation in the presence of extracellular $^{45}\text{Ca}^{2+}$.

Concerning the second major issue of this study, namely, the regulation of Ca^{2+} -dependent insulin secretory events in the islets of ω -3-depleted rats, the essential findings consisted of a somewhat higher release of insulin evoked by 8.3 mM D-glucose, a lesser sensitivity of such a release to the nominal absence of extracellular Ca^{2+} , and, relative to the output of insulin recorded in the presence of 8.3 mM D-glucose, a higher secretory responsiveness to a number of secretagogues in islets from ω -3-depleted rats injected with the MCT:OO compared with the MCT:FO emulsion or in islets from ω -3-depleted rats not injected with any emulsion compared with normal animals.

Last, the measurement of $[\text{Ca}^{2+}]_i$ in dispersed islets from ω -3-depleted rats compared with control animals documented a close analogy between $[\text{Ca}^{2+}]_i$ and insulin output in terms of both the steady-state situation found at 8.3 mM D-glucose and normal extracellular K^+ concentration (5.0 mM) and the time course, as well as relative magnitude of the changes in these variables evoked by a rise in extracellular K^+ concentration.

A possible interpretation of these findings is inspired by the recent observation of a marked impairment of Na^+/K^+ -ATPase activity in islets from ω -3-depleted rats, judged from the time course for $^{86}\text{Rb}^+$ net uptake by islets incubated in the absence or presence of ouabain (8). Indeed, the resulting accumulation of Na^+ in the islet cells and subsequent inhibition of Ca^{2+} extrusion by $\text{Na}^+/\text{Ca}^{2+}$ exchange and mobilization of Ca^{2+} from intracellular organelles may well account for most of the present cationic and secretory data.

First, the increase in the size of the cytosolic Ca^{2+} pool in the islets from ω -3-depleted rats could explain the higher value for the estimated size of the rapidly exchangeable $^{45}\text{Ca}^{2+}$ -labeled cellular pool(s) at so-called isotopic equilibrium. Further support of this proposal resides in the fact that the prior injection of the MCT:FO emulsion, which restores a close-to-normal Na^+/K^+ -ATPase activity in the islets of ω -3-depleted rats, also markedly decreased the size of the $^{45}\text{Ca}^{2+}$ -labeled cellular pool(s) at so-called isotopic equilibrium (Fig. 1).

Second, the cellular accumulation of Na^+ in islets from ω -3-depleted rats would result in the inhibition of forward $\text{Na}^+/\text{Ca}^{2+}$ exchange (Ca^{2+} outflow) or its reversal leading to an increased Ca^{2+} entry by reverse $\text{Na}^+/\text{Ca}^{2+}$ exchange. This could thus account, to some extent at least, for the lower fractional outflow rate of $^{45}\text{Ca}^{2+}$ from the islets of ω -3-depleted rats.

Third, the increase in the cytosolic concentration of Ca^{2+} also may account, at least in part, for the higher release of insulin evoked by 8.3 mM D-glucose in the islets from ω -3-depleted rats compared with normal animals. Likewise, the difference between ω -3-depleted rats injected with the MCT:OO vs. MCT:FO emulsion, in terms of the size of the rapidly exchangeable cellular Ca^{2+} pools at normal extracellular K^+ concentration, as documented in Fig. 1, may account for the higher secretory responsiveness of the islets from ω -3-depleted rats injected with the MCT:OO emulsion, distinct from the MCT:FO emulsion, to a number of insulin secretagogues, as documented in Figs. 2 and 3.

Last, a higher than normal $[\text{Ca}^{2+}]_i$ in the islet cells of ω -3-depleted rats may account for the fact that, at variance with the situation found in islets from normal rats, the release of insulin evoked by 8.3 mM D-glucose in the nominal absence

of extracellular Ca^{2+} was higher in the ω -3-depleted rats than that recorded in the presence of 2.8 mM D-glucose at normal extracellular Ca^{2+} concentration (1.0 mM).

Nevertheless, it should be duly emphasized that the alteration of the ouabain-sensitive modality of K^+ inflow into the islet cells does not represent the sole factor possibly responsible for the alteration of islet secretory behavior in ω -3-depleted rats. For instance, and as already mentioned, the changes in D-glucose metabolism found in the islets of ω -3-depleted rats also may play a role in this respect. Likewise, the modest but significant degree of insulin resistance found in the ω -3-depleted rats may exert a long-term, not rapidly reversible, influence on the secretory activity of islet insulin-producing cells in a manner comparable with that found with a number of other environmental factors (7). Furthermore, in the ω -3-depleted rats, changes in the fatty acid pattern of phospholipids may affect not solely the activity of the Na^+, K^+ -ATPase but also other variables, such as membrane fluidity. Last, and without claiming an exhaustive enumeration, the expression of several genes may be affected in the islet cells of ω -3-depleted rats, as already documented in other cell types (1). Likewise, the present findings should be considered in the framework of prior studies conducted in a number of other cell types and documenting the effects of ω -3-depletion on such items as fatty acid composition of membranes, Na^+, K^+ -ATPase activity, calcium ion channels, signaling pathways, and specific enzymes or receptors (1, 3, 12, 13).

In conclusion, the present findings document significant changes in Ca^{2+} handling and Ca^{2+} -dependent secretory events in islets from ω -3-depleted rats and the qualified correction of some of these defects after intravenous injection of the MCT:FO emulsion to these rats. As such, this study extends knowledge on the multiple consequences of a deficiency in long-chain polyunsaturated ω -3 fatty acids on pancreatic islet function and their possible participation in the perturbation of fuel homeostasis associated with ω -3 depletion.

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