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ORIGINAL PAPER

Direct effects of eicosapentaenoic and docosahexaenoic acids on phospholipid and triglyceride fatty acid pattern, glucose metabolism, ^{86}rubidium net uptake and insulin release in BRIN-BD11 cells

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Abstract The long-term metabolic and functional effects of a dietary deprivation of long-chain polyunsaturated ω3 fatty acids were recently investigated in second-generation ω3-depleted rats. This study represents the first attempt to explore the direct, but not immediate, effects of ω3 fatty acids on insulin-producing cells. For this purpose, BRIN-BD11 cells were cultured for 24 h in the absence or presence of both C20:5ω3 and C22:6ω3 (50 μM each) and, thereafter, examined for their phospholipid and triglyceride fatty acid pattern, and their metabolic, ionic, and secretory responses to D-glucose and/or non-nutrient insulinotropic agents. The prior culture in the presence of the two ω3 fatty acids provoked an enrichment of cell lipids in such ω3 fatty acids, changes in the phospholipid fatty acid pattern of long-chain polyunsaturated ω6 fatty acids as well as saturated and monodesaturated fatty acids, and cell steatosis. It minimized the relative increase in D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation otherwise resulting from an increase in the concentration of the hexose from 1.1 to 11.1 mM. It also minimized the changes in $^{86}Rb^{+}$ net uptake otherwise provoked by rises in D-glucose concentration and decreased the absolute values for insulin output. It is concluded that the major changes in metabolic, cationic, and secretory behavior of the ω3-enriched BRIN-BD11 cells are paradoxically similar to those encountered in pancreatic islets from ω3-depleted rats and, in both cases, possibly attributable to a phenomenon of lipotoxicity.

Keywords BRIN-BD11 cells · Long-chain polyunsaturated ω3 fatty acids · Lipid fatty acid pattern · Glucose metabolism · $^{86}Rb^{+}$ uptake · Insulin release

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Introduction

The long-term effects of a dietary deprivation of long-chain polyunsaturated ω3 fatty acids on enzymatic [1], metabolic [2, 3], ionic [4–6], and secretory [2, 4–6] variables in isolated pancreatic islets were recently investigated in second-generation ω3-depleted rats. Extrapancreatic events including insulin resistance [2, 7], liver steatosis [8, 9], and visceral obesity [10] were also documented in these second-generation ω3-depleted rats, and confirmed in normal rats exposed seven weeks after birth for 3–7 months to an ω3-depleted diet [11]. None of these studies allows an unambiguous distinction between direct versus indirect effects of ω3 fatty acid on pancreatic islet cells. Nevertheless, in these studies, advantage was taken of the intravenous injection of a novel medium-chain triglyceride:fish oil emulsion 60–120 min before sacrifice, to explore rapid changes in the abovementioned variables as resulting from the replenishment of ω3 fatty acids in cell phospholipids of the second-generation ω3 depleted rats.

As a first step to explore the direct, but not immediate, effects of ω3 fatty acids on insulin-producing cells, the experiments of this study deal with changes in phospholipid

and triglyceride fatty acid pattern, glucose metabolism, ^{86}Rb net uptake, and insulin release from BRIN-BD11 cells first cultured 24 h in the absence or presence of both eicosapentaenoic and docosahexaenoic acids (50 μM each). It should not be ignored, however, that BRIN-BD11 cells differ from normal islet cells in such respects as the pattern of glucose metabolism and, hence, the effect of the hexose on cell ATP content [12, 13].

Results

Protein cell content

In the experiments designed to characterize the fatty acid pattern of cell lipids and post 24-h culture is the presence of both eicosapentaenoic and docosahexaenoic acids (50 μM each), the protein content of the BRIN-BD11 cells, expressed per approximately 5×10^7 cells, averaged 19.1 ± 2.2 mg ($n = 3$) as compared ($P > 0.8$) to 18.7 ± 2.0 mg ($n = 3$) in the control cells first cultured in the absence of the two ω3 fatty acids. The measurements made in the former cells represented 102.2 ± 5.5% ($n = 3$; $P > 0.7$) of the paired values found in the control cells. Similarly, in two further experiments designed to measure insulin release, the protein content of the cells, measured in the samples containing about $1.98 \pm 0.07 \times 10^6$ cells, each ($n = 8$), represented in the ω3-enriched cells 106.8 ± 7.1% ($n = 24$; $P > 0.3$) of the mean corresponding values found in the control cells (100.0 ± 2.7%; $n = 24$).

Total fatty acid content of cell lipids

In the control cells not exposed to exogenous long-chain polyunsaturated ω3 fatty acids, the total content of fatty acids in phospholipids and triglycerides averaged 2.89 ± 0.39 and 0.12 ± 0.03 mg/5×10^7 cells, respectively ($n = 3$ in both cases), yielding a paired triglyceride/phospholipid ratio of 4.0 ± 0.6%. The total fatty acid content of phospholipids averaged in the ω3-enriched cells was 136.9 ± 4.3% ($n = 3$; $P > 0.02$) of paired values found in the control cells (Table 1). After exclusion of the long-chain polyunsaturated ω3 fatty acids, however, the remaining fatty acid content of phospholipids in the cells exposed to exogenous C20:5ω3 and C22:6ω3 was no more significantly different from the corresponding values found in the control cells, with a paired ratio between the former and latter cells of 102.7 ± 3.8% ($n = 3$; $P > 0.5$).

Similarly, in the case of triglycerides, the increase in the amount of fatty acids other than long-chain polyunsaturated ω3 fatty acids attributable to exposure of the BRIN-BD11 cells to exogenous C20:5ω3 and C22:6ω3 only represented 34.0 ± 1.7% ($n = 3$) of the paired increase in the total fatty acid content of triglycerides also caused by the presence of C20:5ω3 and C22:6ω3 in the culture medium. As judged from the latter percentage, however, the increase in the amount of fatty acids other than long-chain polyunsaturated ω3 fatty acids in the triglycerides of cells exposed to exogenous C20:5ω3 and C22:6ω3 achieved statistical significance ($P < 0.01$), at variance with the situation found in the phospholipids of the same cells.

Long-chain polyunsaturated ω3 fatty acids content of phospholipids

The absolute value for the weight content of C18:3ω3 in cell phospholipids failed to differ significantly ($P > 0.1$) in control and ω3-enriched cells, with a paired difference between the former and latter cells not exceeding 3.54 ± 1.24 μg/5×10^7 cells ($n = 3$), as compared to an

Table 1 Fatty acid content of cell phospholipids and triglycerides

Cells	Control	ω3-Enriched	ω3-Enriched/control (%)
Phospholipids			
Total (μg/5×10^7 cells)	2,890 ± 387	3,946 ± 516	136.9 ± 4.3[c]
ω3 fatty acids (μg/5×10^7 cells)	218 ± 21	1,212 ± 155[e]	589.2 ± 22.5[e]
Non-ω3 fatty acids (μg/5×10^7 cells)	2,671 ± 367	2,734 ± 362	102.7 ± 3.8
Triglycerides			
Total (μg/5×10^7 cells)	120 ± 31	1159 ± 521	815.2 ± 296.3
ω3 fatty acids (μg/5×10^7 cells)	1 ± 1	680 ± 323	NA
Non-ω3 fatty acids (μg/5×10^7 cells)	119 ± 31	484 ± 202	350.6 ± 105.5

NA not applicable

Mean values (±SEM) refer to 3 separate determinations in all cases

The statistical indices (c: $P < 0.02$; e: $P < 0.005$) refer to the differences between control and ω3-enriched cells (second and third columns) or to the deviation from 100% of the percentages listed in the fourth column

Table 2 Relative weight content of long-chain polyunsaturated $\omega 3$ fatty acids in phospholipids

Cells	Control	$\omega 3$-Enriched	$\omega 3$-Enriched/control (%)
C18:3$\omega 3$ (‰)	3.07 ± 0.74	1.37 ± 0.31	46.0 ± 1.1[f]
C18:4$\omega 3$ (‰)	0.90 ± 0.04	0.27 ± 0.14[c]	29.1 ± 4.7[a]
C20:5$\omega 3$ (‰)	15.31 ± 0.34	100.07 ± 12.44[e]	655.5 ± 86.8[b]
C22:5$\omega 3$ (‰)	17.59 ± 1.76	58.40 ± 8.04[d]	323.3 ± 15.7[e]
C22:6$\omega 3$ (‰)	39.76 ± 3.12	147.36 ± 20.46[d]	381.8 ± 73.9

Mean values (±SEM) refer to 3 separate determinations in all cases

The statistical indices (a: $P < 0.05$; b: $P < 0.025$; c: $P < 0.02$; d: $P < 0.01$; e: $P < 0.005$; f: $P < 0.001$) refer to the differences between control and $\omega 3$-enriched cells (second and third columns) or to the deviation from 100% of the percentages listed in the fourth column

overall mean value of 7.48 ± 1.75 μg/5 × 10^7 cells ($n = 6$). A comparable situation ($P < 0.02$) prevailed in the case of the C18:4$\omega 3$ content of cell phospholipids, with a paired difference between control and $\omega 3$-enriched cells of 1.40 ± 0.17 μg/5 × 10^7 cells ($n = 3$) and an overall mean value of 1.94 ± 0.45 μg/5 × 10^7 cells ($n = 6$). The weight percentages of C18:3$\omega 3$ and C18:4$\omega 3$ were also both decreased, in the phospholipids of cells exposed to exogenous $\omega 3$ fatty acids (Table 2). On the contrary, the weight percentages of C20:5$\omega 3$, C22:5$\omega 3$ and C22:6$\omega 3$ were strikingly increased in the same cells. The relative magnitude of such an increase was higher ($P < 0.01$) for C20:5$\omega 3$ (multiplication factor 6.6 ± 0.9; $n = 3$) than for C22:5$\omega 3$ and C22:6$\omega 3$ (multiplication factor 3.5 ± 0.4; $n = 6$). In absolute terms, the total content of phospholipids in $\omega 3$ fatty acids was increased ($P < 0.005$) from a control value of 0.22 ± 0.02 to 1.21 ± 0.16 mg/5 × 10^7 cells ($n = 3$ in both cases), yielding a multiplication factor of 5.5 ± 0.3 ($n = 3$; $P < 0.005$). The relative contribution of C20:5$\omega 3$, C22:5$\omega 3$ and C22:6$\omega 3$ to the total increment of these three fatty acids averaged 35.3 ± 5.4, 18.1 ± 3.0 and 46.6 ± 8.3% ($n = 3$ in all cases), respectively.

When detectable amount of both C18:3$\omega 3$ and C18:4$\omega 3$ were found in the phospholipids, the C18:4$\omega 3$/C18:3$\omega 3$ ratio failed to differ significantly between the control cells and those exposed to exogenous $\omega 3$ fatty acids, with the ratio found in the latter cells averaging 97.0 ± 13.3% ($n = 2$) of the paired values found in control cells (Table 3). Similarly, neither the C22:6$\omega 3$/C20:5$\omega 3$ ratio nor the C22:5$\omega 3$/C22:6$\omega 3$ ratio differed significantly between the control cells and $\omega 3$-enriched cells (Table 3). There was a trend, however, toward a lower value for the C22:6$\omega 3$/C20:5$\omega 3$ ratio in the cells exposed to exogenous $\omega 3$ fatty acids than in the control cells, with a paired ratio of 62.9 ± 17.6% ($n = 3$) between the former and latter cells.

Table 3 Paired ratios between selected long-chain polyunsaturated $\omega 3$ fatty acids in phospholipids

Cells	Control	$\omega 3$-Enriched	P
C18:4$\omega 3$/C18:3$\omega 3$	0.250 ± 0.001 (2)	0.242 ± 0.032 (2)	>0.8
C22:6$\omega 3$/C20:5$\omega 3$	2.60 ± 0.22 (3)	1.56 ± 0.36 (3)	<0.1
C22:5$\omega 3$/C22:6$\omega 3$	0.440 ± 0.008 (3)	0.432 ± 0.130 (3)	>0.95

Long-chain polyunsaturated $\omega 6$ fatty acids content of phospholipids

In both the control and $\omega 3$-enriched cells, the most abundant long-chain polyunsaturated $\omega 6$ fatty acids in phospholipids were C18:2$\omega 6$ followed by C20:4$\omega 6$ and C20:3$\omega 6$. The relative contributions of C18:2$\omega 6$, C18:3$\omega 6$, C20:3$\omega 6$, C20:4$\omega 6$ and C22:4$\omega 6$ were all significantly lower in $\omega 3$-enriched cells, than in control ones (Table 4) with such a decrease failing, however, to achieve statistical significance ($P < 0.08$) in the case of C20:2$\omega 6$. The lower relative contribution of long-chain polyunsaturated $\omega 6$ fatty acids in phospholipids of $\omega 3$-enriched cells could not be merely attributed to the accumulation of long-chain polyunsaturated $\omega 3$ fatty acids in the same cells. Indeed, the ratio between the relative abundance of a given long chain polyunsaturated $\omega 6$ fatty acids in $\omega 3$-enriched/control cells was not comparable in all cases. It ranged between the extreme values ($P < 0.02$) of 35.5 ± 4.0% (C18:3$\omega 6$) and 73.8 ± 7.3% (C20:2$\omega 6$).

While the conversion from C18:2$\omega 6$ to C20:2$\omega 6$, as judged from the C20:2$\omega 6$/C18:2$\omega 6$ ratio, appeared facilitated in the $\omega 3$-enriched cells, the opposite situation prevailed for the conversion from C18:2$\omega 6$ to C18:3$\omega 6$ (Table 5). The following two steps in the chain of reactions leading from linoleate to docosatetraenoate, however, yielded higher mean C20:3$\omega 6$/C18:3$\omega 6$ and C20:4$\omega 6$/C20:3$\omega 6$ ratios in $\omega 3$-enriched cells than in control ones.

Table 4 Relative weight content of long-chain polyunsaturated $\omega 6$ fatty acids in phospholipids

Cells	Control	$\omega 3$-Enriched	$\omega 3$-Enriched/control (%)
C18:2$\omega 6$ (‰)	108.7 ± 12.5	62.4 ± 3.6[a]	57.7 ± 1.3[e]
C18:3$\omega 6$ (‰)	3.11 ± 0.10	1.10 ± 0.09[f]	35.5 ± 4.0[e]
C20:2$\omega 6$ (‰)	2.20 ± 0.26	1.58 ± 0.05	73.8 ± 7.3
C20:3$\omega 6$ (‰)	14.7 ± 0.5	7.6 ± 0.3[f]	51.5 ± 1.7[e]
C20:4$\omega 6$ (‰)	60.6 ± 4.3	42.7 ± 3.5[a]	70.3 ± 1.5[e]
C22:4$\omega 6$ (‰)	1.93 ± 0.24	1.17 ± 0.08[a]	61.3 ± 3.4[d]

Mean values (±SEM) refer to 3 separate determinations in all cases

The statistical indices (a: $P < 0.05$; c: $P < 0.02$; d: $P < 0.01$; e: $P < 0.005$; f: $P < 0.001$) refer to the differences between control and $\omega 3$-enriched cells (second and third columns) or to the deviation from 100% of the percentages listed in the fourth column

Table 5 Paired ratios between selected long-chain polyunsaturated ω6 fatty acids in phospholipids

Cells	Control	ω3-Enriched	ω3-Enriched/control (%)
C20:2ω6/C18:2ω6 (‰)	20.2 ± 0.2	25.8 ± 2.0[a]	127.4 ± 10.0
C18:3ω6/C18:2ω6 (‰)	29.8 ± 5.0	17.6 ± 0.3	61.8 ± 8.1[a]
C20:3ω6/C18:3ω6	4.72 ± 0.00	7.06 ± 0.95	149.6 ± 20.1
C20:4ω6/C20:3ω6	4.12 ± 0.16	5.62 ± 0.23[d]	136.4 ± 0.8[f]
C22:4ω6/C20:4ω6 (‰)	31.5 ± 1.5	27.5 ± 0.4	87.7 ± 4.9

Mean values (±SEM) refer to 3 separate determinations in all cases

The statistical indices (a: $P < 0.05$; d: $P < 0.01$; f: $P < 0.001$) refer to the differences between control and ω3-enriched cells (second and third columns) or to the deviation from 100% of the percentages listed in the fourth column

Such a difference failed to reach statistical significance ($P < 0.07$) in the case of the C20:3ω6/C18:3ω6 ratio. Moreover, the mean C22:4ω6/C20:4ω6 was again lower, albeit not quite significantly so ($P < 0.06$), in ω3-enriched cells than in control ones.

Saturated and mono-desaturated fatty acid content of phospholipids

For the sake of uniformity, the weight contribution of saturated and mono-desaturated fatty acids in the phospholipids of both control and ω3-enriched cells are expressed in Table 6 relative to the total fatty acid content of phospholipids. The data presented in this table may be misleading, however. For instance, in the case of the most abundant fatty acid among all saturated and mono-desaturated fatty acids, i.e., C16:0, the results listed in Table 6 suggest that the relative contribution of C16:0 alone represented, in the ω3-enriched cells, 93.4 ± 1.0% ($n = 3$); $P < 0.025$), of the corresponding value found in the same experiment(s) in control cells. Yet, if the amount of long-chain polyunsaturated ω3 fatty acids present in phospholipids is ignored, the weight percentage of C16:0 in the ω3-enriched cells (37.0 ± 0.9%; $n = 3$) was higher ($P < 0.01$) than that of C16:0 in the control cells (29.8 ± 1.1%; $n = 3$) when the ω3-enriched cells/control cells ratio was 124.5 ± 2.1% ($n = 3$; $P < 0.01$ vs. 100%). Nevertheless the data listed in Table 6 duly indicate that the accumulation of long-chain polyunsaturated ω3 fatty acids in the cells exposed to exogenous C20:5ω3 and C22:6ω3 does not represent the sole factor responsible for the difference in the relative contribution of any given fatty acid to phospholipids of control versus ω3-enriched cells. Indeed, the ω3-enriched/control ratio for the relative weight was quite variable, ranging between the extreme mean values of 40.7 ± 2.1% (C20:1ω9) and 94.8 ± 24% (C18:0).

Table 6 Relative weight content of saturated and mono-desaturated fatty acids in phospholipids

Cells	Control	ω3-Enriched	ω3-Enriched/control (%)
C14:0 (‰)	23.2 ± 0.6	20.0 ± 1.4	85.9 ± 3.9
C16:0 (‰)	274 ± 9	256 ± 6	93.4 ± 1.0[b]
C16:1ω7 (‰)	45.9 ± 0.9	20.6 ± 2.5[f]	44.6 ± 4.9[d]
C18:0 (‰)	151 ± 1	144 ± 3	94.8 ± 2.4
C18:1ω9 (‰)	214 ± 2	120 ± 3[f]	55.7 ± 1.0[f]
C20:0 (‰)	3.63 ± 0.12	2.40 ± 0.12[e]	66.8 ± 5.8[a]
C20:1ω9 (‰)	3.55 ± 0.19	1.44 ± 0.01[f]	40.7 ± 2.1[e]
C22:0 (‰)	3.64 ± 0.02	2.64 ± 0.13[e]	72.5 ± 3.4[c]
C22:1ω9 (‰)	1.41 ± 0.11	0.95 ± 0.09[a]	67.2 ± 1.4[e]
C24:0 (‰)	10.2 ± 0.0	8.3 ± 0.2[f]	81.8 ± 1.7[d]

Mean values (±SEM) refer to 3 separate determinations in all cases

The statistical indices (a: $P < 0.05$; b: $P < 0.025$; c: $P < 0.02$; d: $P < 0.01$; e: $P < 0.005$; f: $P < 0.001$) refer to the differences between control and ω3-enriched cells (second and third columns) or to the deviation from 100% of the percentages listed in the fourth column

The mean weight relative contribution of all the saturated and mono-desaturated fatty acids in phospholipids was lower in the ω3-enriched cells than in control ones (Table 6). Such a difference achieved statistical significance in most cases. Even C12:0 was detected in two out of three samples of control cells (relative contribution: 0.53 ± 0.05 per thousand; $n = 2$), while it was never such a case in ω3-enriched cells. In the case of C14:0, the paired difference in its relative contribution (3.21 ± 0.81 per thousand; $n = 3$) between control and ω3-enriched cells almost achieved statistical significance ($P < 0.06$). Similar was also the case in respect of the difference between the mean values for the relative weight content of C18:0 ($P < 0.06$).

Further information concerning the saturated and mono-desaturated fatty acids of phospholipids was gained by computing the paired ratio between distinct fatty acids engaged in reactions catalyzed by either desaturase or elongase (Table 7).

The C16:ω7/C16:0 and C18:1ω9/C18:0 ratios in phospholipids were both much lower in ω3-enriched cells than in control ones, suggesting decreased Δ9 desaturase activity in

Table 7 Paired ratios between selected saturated and mono-desaturated fatty acids in phospholipids

Cells	Control	ω3-Enriched	ω3-Enriched/control (%)
C16:1ω7/C16:0 (%)	16.8 ± 0.8	8.1 ± 1.1[e]	47.7 ± 4.9[d]
C18:1ω9/C18:0	1.42 ± 0.00	0.83 ± 0.03[f]	58.8 ± 2.2[e]
C16:0/C14:0	11.9 ± 0.7	13.0 ± 1.3	111.4 ± 5.0
(C18:0 + C18:1ω9)/(C16:0 + C16:1ω7)	1.14 ± 0.04	0.95 ± 0.02[c]	83.4 ± 2.2[c]
C20:0/C18:0 (%)	2.40 ± 0.06	1.68 ± 0.06[f]	70.0 ± 4.1[c]
C22:0/C20:0	1.00 ± 0.04	1.09 ± 0.01	109.3 ± 4.2
C24:0/C22:0	2.81 ± 0.02	3.17 ± 0.08[d]	113.1 ± 2.9[a]
C20:1ω9/C18:1ω9 (%)	1.66 ± 0.07	1.20 ± 0.02[e]	73.2 ± 4.6[a]
C22:1ω9/C20:1ω9	0.40 ± 0.05	0.66 ± 0.07[a]	165.7 ± 5.9[d]

Mean values (±SEM) refer to 3 separate determinations in all cases

The statistical indices (a: $P < 0.05$; c: $P < 0.02$; d: $P < 0.01$; e: $P < 0.005$; f: $P < 0.001$) refer to the differences between control and ω3-enriched cells (second and third columns) or to the deviation from 100% of the percentages listed in the fourth column

the former versus latter cells (Table 7). The paired ratios between saturated fatty acids were, as a rule, not significantly different in the phospholipids of control and ω3-enriched cells (Table 7). Similar were the cases in respect of the C16:0/C14:0 and C22:0/C20:0 ratios. The two most obvious changes consisted in a significant decrease of the (C18:0 + C18:1ω9)/(C16:0 + C16:1ω7) and C20:0/C18:0 ratios in the ω3-enriched cells. A minor, but significant, increase in the ω3-enriched cells was only observed in the cases of the C24:0/C22:0 ratio. Last, in the pathway leading to the generation of nervonic acid from oleic acid, the C20:1ω9/C18:1ω9 ratio was also significantly decreased in the ω3-enriched cells, while a mirror image prevailed in the case of the C22:1ω9/C20:1ω9 ratio.

Fatty acid pattern of triglycerides

A sizeable amount of a long-chain polyunsaturated ω3 fatty acid in triglycerides was only detected once in the control cells (2.7 μg/5 × 10^7 cells of C22:6ω3). In the ω3-enriched cells, when detected, the sole C18:3ω3 content of triglycerides already amounted to 6.3 ± 0.4 μg/5 × 10^7 cells ($n = 2$). The absolute values for C20:5ω3, C22:5ω3, and C22:6ω3, each considered separately, averaged in the triglycerides of ω3-enriched cells no less than 225.0 ± 87.5 μg/10^5 cells ($n = 9$). The weight percentage of C20:5ω3, C22:5ω3, and C22:6ω3 in those triglycerides yielded respective mean values of 6.6 ± 0.4, 13.1 ± 0.3 and 32.2 ± 7.2% ($n = 3$ in each case).

In the triglycerides of ω3-enriched cells, both the C22:6ω3/C20:5ω3 and C22:5ω3/C22:6ω3 ratios displayed rather great individual variability, with respective variation coefficient (SD/mean) of no less than 35.8 and 51.5%. Nevertheless, in both cases, there was a close parallelism with the individual values for the same ratio in the phospholipids of the same cells. Indeed, the triglyceride/phospholipid ratio, as computed in each cell sample, yielded corresponding variation coefficients of no more than 5.3 ± 4.6%, with a correlation coefficient between phospholipid and triglyceride data amounting to 0.984 ($n = 6$; $P < 0.001$). In this respect, the triglyceride/phospholipid ratio was close to unity (1.085 ± 0.005; $n = 3$) in the case of the C22:5ω3/C22:6ω3 ratio, while averaging 3.145 ± 0.180 ($n = 3$) in the case of C22:6ω3/C20:5ω3 ratio.

The sole long-chain polyunsaturated ω6 fatty acid detected in the triglycerides of control cells was C18:2ω6. Its relative content averaged 1.3 ± 0.6% ($n = 3$). In the ω3-enriched cells, however, sizeable amount of several long-chain polyunsaturated ω6 fatty acids were found. Relative to the total fatty acids content of triglycerides, at the exclusion of long-chain polyunsaturated ω3 fatty acids, their relative contribution averaged 8.2 ± 2.6% for C18:2ω6, 0.2 ± 0.1% for C20:2ω6, 0.9 ± 0.4% for C20:3ω6, 1.1 ± 0.5% for C20:4ω6 and 0.1 ± 0.1% for C22:4ω6.

In the triglyceride of control cells, the most abundant saturated and mono-desaturated fatty acids were C16:0 (49.0 ± 3.1%) and C18:0 (19.5 ± 1.7%), followed by C18:1ω9 (9.9 ± 0.6%), C14:0 (7.7 ± 0.9%), C12:0 (4.7 ± 1.5%); C16:1ω7 (4.3 ± 1.7%) and C20:0 (1.0 ± 0.5%). The weight percentages of each of these fatty acids failed to differ significantly in control and ω3-enriched cells, when expressed relative to the total content of triglyceride fatty acids, at the exclusion of long-chain polyunsaturated ω3 fatty acids. For instance, for the four most abundant saturated and monodesaturated fatty acids mentioned above, such a weight percentage averaged, in the ω3-enriched cells, 95.2 ± 5.0% ($n = 12$; $P > 0.3$) of the paired values found within the same experiment in control cells. However and as already alluded to, the absolute amount of the saturated and monodesaturated fatty acids was, as a rule, higher in the ω3-enriched cells than in the

control ones. For instance, in the case of C12:0, C14:0, C16:0, C18:0 and C20:0, such an absolute amount represented in the ω3-enriched cells 229.6 ± 41.2% ($n = 15$; $P < 0.01$) of the paired value found, within the same experiment, in the control cells. A small amount of C24:0 was, on occasion, detected in the triglycerides of ω3-enriched cells. Its concentration, relative to the total amount of triglyceride fatty acids at the exclusion of long-chain polyunsaturated ω3 fatty acids, did not exceed 0.3 ± 0.1% ($n = 3$).

The C16:1ω7/C16:0 and C18:1ω9/C18:0 ratios in triglycerides also failed to differ significantly in control and ω3-enriched cells, with an ω3-enriched/control paired ratio averaging 100.6 ± 19.2% ($n = 6$; $P > 0.975$).

Glucose metabolism

In both control and ω3-enriched cells, the rise in D-glucose concentration from 1.1 to 11.1 mM augmented ($P > 0.001$) the generation of ^{3}HOH from D-[5-^{3}H]glucose. In absolute terms, such an increase failed to differ significantly ($P > 0.3$) in control cells (+130.0 ± 17.5 pmol/10^3 cells per 90 min; df = 30) and ω3-enriched cells (+111.7 ± 11.6 pmol/10^3 cells per 90 min; df = 35). In relative terms, however, the increment in D-[5-^{3}H]glucose utilization attributable to the rise in hexose concentration, was less pronounced ($P < 0.025$) in ω3-enriched cells than in control cells (Table 8). As judged from the absolute values listed in Table 8, the generation of ^{3}HOH from D-[5-^{3}H]glucose was indeed significantly higher in ω3-enriched cells than in control cells, when exposed to 1.1 mM D-glucose, but no longer so when the concentration of the hexose was raised to 11.1 mM.

When all the results were expressed relative to the mean value recorded within the same experiment(s) in the control cells exposed to the same hexose concentration, the data found in ω3-enriched cells incubated at 1.1 and 11.1 mM D-glucose, respectively, averaged, however, 151.7 ± 6.0% (df = 34; $P < 0.001$) and 139.3 ± 12.2% (df = 35; $P < 0.005$). The latter two percentages failed to differ significantly from one another ($P > 0.3$), yielding an overall mean value of 145.4 ± 6.9% (df = 69; $P < 0.001$).

In the control cells, the rise in D-glucose concentration also augmented the generation of $^{14}CO_2$ from D-[U-^{14}C]glucose. This was no longer the case, however, in the ω3-enriched cells. Thus, whether judged from the absolute value for the increase in D-[U-^{14}C]glucose oxidation (1.26 ± 1.01 pmol/10^3 cells per 90 min; df = 33) or the 11.1 mM/1.1 mM ratio for $^{14}CO_2$ output (106.4 ± 5.3%; df = 35), the ω3-enriched cells failed to display any significant oxidative response to the rise in hexose concentration. Moreover, these two mean values were both significantly lower ($P < 0.02$ or less) than those recorded in the control cells, i.e., 5.60 ± 1.43 pmol/10^3 cells per 90 min ($P < 0.001$; df = 29) and 141.3 ± 9.9% ($P < 0.001$; df = 33). In other words, while the rate of D-[U-^{14}C]glucose oxidation by the ω3-enriched cells averaged at 1.1 mM D-glucose 140.6 ± 5.5% ($P < 0.001$; $n = 19$) of that recorded within the same experiment(s) and at the same hexose concentration in the control cells (100.0 ± 7.6%; $n = 17$), it only represented 105.7 ± 3.6% ($P > 0.3$; $n = 18$) of the corresponding control value (100.0 ± 4.3%; $n = 16$) in the presence of 11.1 mM D-glucose. Such a difference (140.6 ± 9.3% vs. 105.7 ± 5.7%; df = 34 and 32) remained highly significant ($P < 0.005$) even when taking into account the dispersion of results at each D-glucose concentration in both cell types.

As expected, in the BRIN-BD11 tumoral control cells exposed to 11.1 mM D-glucose the paired ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization only represented 76.8 ± 5.5% ($P < 0.05$; $n = 16$) of the mean corresponding value(s) found within the same experiment(s) at 1.1 mM D-glucose (100.0 ± 8.7%; $n = 17$). A comparable situation prevailed in the ω3-enriched cells (Table 8). Thus, neither the absolute values for such a ratio, whether at 1.1 or 11.1 mM D-glucose, nor the 11.1 mM/

Table 8 Glucose metabolism

Cells	Control	ω3-Enriched	P
D-[5-^{3}H]glucose utilization (pmol/10^3 cells per 90 min)			
D-Glucose 1.1 mM	127.1 ± 11.7 (17)	187.9 ± 12.1 (19)	<0.005
D-Glucose 11.1 mM	256.8 ± 34.0 (17)	295.8 ± 22.7 (20)	>0.3
11.1/1.1 mM ratio (%)	187.8 ± 11.7 (*32*)	157.8 ± 6.4 (*37*)	<0.025
D-[U-^{14}C]glucose oxidation (pmol/10^3 cells per 90 min)			
D-Glucose 1.1 mM	13.3 ± 1.3 (17)	18.5 ± 1.1 (19)	<0.005
D-Glucose 11.1 mM	19.1 ± 1.5 (16)	20.0 ± 1.3 (18)	>0.6
11.1/1.1 mM ratio (%)	141.3 ± 9.9 (*31*)	106.4 ± 5.3 (*35*)	<0.005
D-[U-^{14}C]glucose oxidation/D-[5-^{3}H]glucose utilization (%)			
D-Glucose 1.1 mM	11.3 ± 1.2 (17)	10.1 ± 0.4 (19)	>0.3
D-Glucose 11.1 mM	8.9 ± 1.0 (16)	7.0 ± 0.5 (18)	>0.08
11.1/1.1 mM ratio (%)	76.8 ± 10.5 (*31*)	69.4 ± 5.7 (*35*)	>0.50

The italicized values correspond to df (degree of freedom)

1.1 mM ratio for the same variable differed significantly in control and ω3-enriched cells. Even when ignoring the dispersion of the results collected at 1.1 mM D-glucose, the latter ratio indeed averaged 76.8 ± 5.5% ($n = 16$) and 69.4 ± 4.2% ($n = 18$) in the control and ω3-enriched cells, respectively ($P > 0.29$). At the most, there was a trend ($P < 0.07$) for the values recorded in ω3-enriched cells to be somewhat lower (88.2 ± 3.8%; $n = 37$) than those recorded within the same experiments and at the same hexose concentration (1.1 or 11.1 mM) in the control cells (100.0 ± 5.0%; $n = 33$).

$^{86}Rb^{+}$ net uptake

After 60-min incubation in the sole presence of 1.1 mM D-glucose, the net uptake of $^{86}Rb^{+}$ by the BRIN-BD11 cells averaged, in the ω3-enriched cells, 106.8 ± 4.8% ($P > 0.2$; $n = 44$) of the mean corresponding value found within the same experiment(s) in the control cells (100.0 ± 3.5%; $n = 46$). The corresponding absolute values, expressed as K^{+} equivalent, were 119.0 ± 6.5 fmol/cell ($n = 44$) in the ω3-enriched cells, as compared ($P > 0.65$) to 115.4 ± 6.5 fmol/cell ($n = 46$) in the control cells.

The net uptake of $^{86}Rb^{+}$ by BRIN-BD11 cells exposed to 1.1 mM D-glucose was first measured after 60-min incubation in the absence or presence of ouabain (0.1 mM). In this set of experiments, the reference net uptake of $^{86}Rb^{+}$ measured in the absence of ouabain was somewhat higher ($P < 0.02$) in the ω3-enriched cells than in the control ones (Fig. 1). Such a difference persisted ($P < 0.01$) in the presence of ouabain. Expressed relative to the mean reference values recorded within the same experiment(s), the ouabain-resistant net uptake of $^{86}Rb^{+}$ failed, however, to differ significantly ($P > 0.35$) in the ω3-enriched cells (67.3 ± 3.4%; $n = 23$) and control cells (61.8 ± 5.4%; $n = 24$). The absolute values for the ouabain-sensitive net uptake of $^{86}Rb^{+}$ also failed to differ significantly ($P > 0.7$) in the ω3-enriched cells (49.7 ± 6.9 fmol/cell; df = 41) and control cells (52.9 ± 9.0 fmol/cell; df = 43).

The effect of increasing concentrations of D-glucose upon $^{86}Rb^{+}$ net uptake was also examined. As illustrated in Fig. 2, in the control cells, the net uptake of $^{86}Rb^{+}$ averaged in the absence of D-glucose and presence of 11.1 mM D-glucose, respectively, recorded 82.6 ± 5.9% ($P < 0.04$; $n = 24$) and 49.2 ± 4.4% ($P < 0.001$; $n = 38$) of the mean reference value found within the same experiment(s) in the presence of 1.1 mM D-glucose (100.0 ± 5.5%; $n = 23$). In the ω3-enriched cells, the values recorded in the absence of D-glucose and presence of 11.1 mM D-glucose represented, respectively, 109.1 ± 4.9% ($P > 0.2$; $n = 24$) and 66.5 ± 5.2% ($P < 0.001$; $n = 38$) of the corresponding reference values found at 1.1 mM D-glucose (100.0 ± 5.2%; $n = 22$). As judged from these values, the $^{86}Rb^{+}$ net uptake recorded in either the absence of exogenous nutrient or presence of a high concentration of D-glucose, expressed relative to that found at 1.1 mM D-glucose, were both higher ($P < 0.02$ or less) in the ω3-enriched cells than in control ones (Fig. 2).

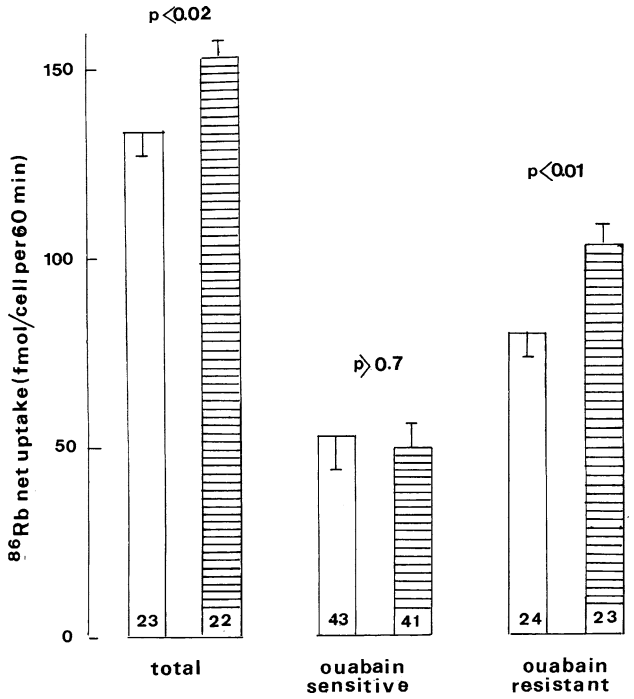

Fig. 1 Absolute values for $^{86}Rb^{+}$ net uptake by control (*open columns*) and ω3-enriched (*hatched columns*) BRIN-BD11 cells incubated in the absence (total) or presence (ouabain-resistant) of 0.1 mM ouabain; mean values (±SEM) refer to the number of separate determinations indicated at the bottom of each column. The ouabain-sensitive $^{86}Rb^{+}$ net uptake was calculated from the differences between the mean values for total uptake and ouabain-resistant uptake within each experiment; mean values (±SEM) refer to the degree of freedom indicated at the bottom of each column

Insulin release

When incubated for 60 min in the presence of 1.1 mM D-glucose, the release of insulin from the control cells averaged 84.3 ± 5.4 μU/2 × 10^{6} cells ($n = 6$). Relative to cell protein and insulin content, respectively, this corresponded to 208.5 ± 31.6 μU/mg ($n = 6$) and 14.6 ± 0.5% ($n = 6$).

As illustrated in Table 9, the secretory responsiveness to nutrient and non-nutrient secretagogues of the ω3-enriched cells was virtually identical to that of control cells. In the absence of any insulinotropic agent, the release of insulin averaged 81.1 ± 3.6% ($P < 0.001$; $n = 12$) of the mean reference values recorded within the same experiments in the presence of 1.1 mM D-glucose (100.0 ± 2.5%;

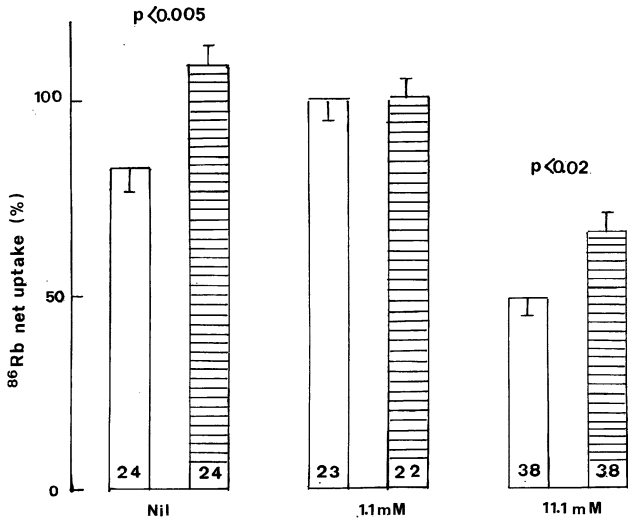

Fig. 2 Effects of increasing concentrations of D-glucose upon $^{86}Rb^{+}$ net uptake by control (*open columns*) and ω3-enriched (*hatched columns*) BRIN-BD11 cells. All the results are expressed relative to the mean values found within the same experiments and in the same type of cells (control or ω3-enriched cells) incubated at 1.1 mM D-glucose. Mean values (±SEM) refer to the number of separate determinations indicated at the bottom of each column, and are shown together with the significance of differences between control and ω3-enriched cells

$n = 12$). A rise in D-glucose concentration to 11.1 mM D-glucose failed to further increase insulin output, which averaged 99.0 ± 3.4% ($P > 0.8$; $n = 12$) of the same reference value. Theophylline (1.4 mM) augmented ($P < 0.001$) the release of insulin evoked by 11.1 mM D-glucose to 131.5 ± 3.9% ($n = 12$) of the reference value otherwise found in the sole presence of 1.1 mM D-glucose.

Under these four experimental conditions, the absolute values for insulin output were lower, however, in the ω3-enriched cells than in control cells. It indeed averaged in the former cells 77.0 ± 3.9% ($P < 0.001$; $n = 24$) of the mean corresponding values found under the same experimental conditions and within the same experiments in the control cells (100.0 ± 2.2%; $n = 24$). Similarly, when the output of insulin was expressed relative to the protein content of the cells within each individual sample, the values recorded in the ω3-enriched cells averaged 73.1 ± 1.7% ($P < 0.001$; $n = 24$) of the mean corresponding values found under the same experimental conditions and within the same experiments in the control cells (100.0 ± 1.6%; $n = 24$).

The relative extent of the decrease in insulin output attributable to the prior culture for 24 h in the presence of both eicosapentaenoic and docosahexaenoic acid thus failed to differ significantly ($P > 0.4$), whether judged from the absolute values for insulin output (23.0 ± 4.5%; df = 46) or ratio between insulin release and cell protein content (26.9 ± 2.3%; df = 46).

Essentially, comparable results were obtained when each individual value for insulin output was expressed relative to the insulin content of cells in the same sample. For instance, in the control cells, such a ratio progressively increased ($r = 0.5312$; df = 21; $P < 0.01$) from 12.4 ± 1.4% ($n = 5$) in the absence of glucose to 14.6 ± 0.5% ($n = 6$) in the presence of 1.1 mM D-glucose, 15.6 ± 1.1% ($n = 6$) in the presence of 11.1 mM D-glucose, and 17.1 ± 1.6% ($n = 6$) in the presence of both 11.1 mM D-glucose and 1.4 mM theophylline. Similarly, in the ω3-enriched cells, such a ratio also progressively increased ($r = 0.4210$; df = 22; $P < 0.05$) under the same four experimental conditions. Moreover, the output/content ratio remained lower in the ω3-enriched cells than in the control ones, averaging in the former cells 83.1 ± 7.3% ($n = 24$; $P < 0.03$) of the mean corresponding control values found under the same experimental conditions within the same experiment(s) (100.0 ± 3.3%; $n = 23$). The insulin content of the cells failed to differ significantly ($P > 0.6$), however, in the control cells (602 ± 25 μU/2 × 10^6 cells; $n = 23$) and ω3-enriched cells (632 ± 58 μU/2 × 10^6 cells; $n = 24$).

Discussion

This study deals with the direct, but not immediate, effects of both eicosapentaenoic and docosahexaenoic acids upon selected variables in BRIN-BD11 cells. It should be stressed, therefore, that the time course for such effects was not investigated, the BRIN-BD11 cells being always first cultured for 24 h in the absence or presence of these two

Table 9 Insulin release

Cells	Control	ω3-Enriched	*P*
No glucose	86.1 ± 3.5 (6)[a]	76.1 ± 5.9 (6)	>0.15
D-Glucose 1.1 mM	100.0 ± 4.8 (6)	100.0 ± 2.3 (6)	
D-Glucose 11.1 mM	99.5 ± 4.9 (6)	98.5 ± 5.0 (6)	>0.8
D-Glucose 11.1 mM + theophyline 1.4 mM	130.3 ± 7.1 (6)	132.6 ± 4.1 (6)	>0.7

[a] Results expressed in percent of the mean value recorded within the same experiment(s) and in the same type of cells (control or ω3-enriched) in the sole presence of 1.1 mM D-glucose

long-chain polyunsaturated ω3 fatty acids before measuring their phospholipid and triglyceride fatty acid pattern, glucose metabolism, ^{86}Rb net uptake, and insulin release. Similarly, it should be underlined that the study was conducted in tumoral insulin-producing cells of the BRIN-BD11 line and not in pancreatic islets or dispersed islet cells prepared from normal rats. In these two respects, this study should be considered, as already indicated in the introduction of this article, as a first step in investigating the direct effects on pancreatic islet cells of ω3-fatty acid supply or deprivation.

The two long-chain polyunsaturated ω3-fatty acids added to the culture medium were efficiently incorporated in both the phospholipids and triglycerides of BRIN-BD11 cells exposed to the ω3 fatty acids for 24 h. Despite the fact that C20:5ω3 and C22:6ω3 were present at the same concentration in the culture medium, the enrichment of both phospholipids and triglycerides in C22:6ω3 was higher ($P < 0.05$) than that found for C20:5ω3, with a paired C22:6ω3/C20:5ω3 ratio amounting to 313.3 ± 80.8% ($n = 6$). In relative terms, such a difference was more pronounced ($P < 0.05$) in triglycerides than in phospholipids, with a paired triglyceride/phospholipid ratio averaging 3.57 ± 0.43 ($n = 3$). A comparable preferential enrichment of liver phospholipids in C22:6ω3 relative to C20:5ω3 was recently documented in second-generation ω3-depleted rats examined 60 min after the intravenous injection of a medium-chain triglyceride:fish oil emulsion [14].

For purpose of comparison, in a prior study [15], the plasma concentration of unesterified C20:5ω3 and C22:6ω3 averaged, in normal male rats, respectively 11.3 ± 2.5 and 81.0 ± 13.7 μM ($n = 9$ in both cases), and was increased ($P < 0.05$ or less) to 95.8 ± 19.9 and 148.5 ± 31.8 μM ($n = 5$ in both cases) 60 min after the intravenous injection of the ω3-rich medium-chain triglyceride:fish oil emulsion. The mean increment in the plasma concentration provoked by the latter injection failed to differ significantly ($P > 0.6$) in the case of C20:5ω3 and C22:6ω3, with an overall mean value of 76.0 ± 16.3 μM (df = 24; $P < 0.001$). In the same study, the plasma concentration of C20:5ω3 and C22:6ω3 remained in all of the 15 second-generation ω3-depleted rats below the limit of detection, while averaging, respectively, 57.8 ± 7.3 and 65.4 ± 5.5 μM ($n = 12$ in both cases) 60 min after the intravenous injection of the same ω3-rich emulsion. The latter two increments failed to differ significantly from one another ($P > 0.4$) and their overall mean value (61.6 ± 4.5 μM; $n = 24$) failed to differ significantly ($P > 0.4$) from the increment in the plasma concentration of unesterified C20:5ω3 and C22:6ω3 found in the normal rats injected with the same emulsion. Such increments were also close to the concentrations of C20:5ω3 and C22:6ω3 used for the experiments in this study. The phospholipid C20:5ω3 and C22:6ω3 relative weight contents of the control BRIN-BD11 cells failed to differ significantly from that previously measured in cultured bovine aortic endothelial cells [16], being in both cases lower for C20:5ω3 (1.2 ± 0.3%) than for C22:6ω3 (4.4 ± 0.5%). Similarly, the mean increment in such relative contents provoked by exposure of the cells to exogenous long-chain polyunsaturated ω3 fatty acids was comparable in the BRIN-BD11 cells and the bovine aortic endothelial cells, averaging, respectively, in these two cell types at 9.6 ± 1.1 and 8.9 ± 2.2%. When allowance was made for the reciprocal effects of C20:5ω3 and C22:6ω3 upon their respective incorporation in the phospholipids of bovine aortic endothelial cells, however, the phospholipid relative weight content of C20:5ω3 and C22:6ω3 in the ω3-enriched BRIN-BD11 cells were, as expected from the difference in the length of the culture period, higher than those recorded in the aortic endothelial cells after only 4 h of incubation, in which case it averaged no more than 56.5 ± 4.2% of the corresponding value found for the same ω3 fatty acid in the BRIN-BD11 cells.

The fatty acid pattern of phospholipids and triglycerides differed in the ω3-enriched cells as compared with control BRIN-BD11 cells in other respects than their content in long-chain polyunsaturated ω3-fatty acids. For instance, in the phospholipids, the C18:3ω6/C18:2ω6 and C22:4ω6/C20:4ω6 ratios were both lower in ω3-enriched cells than in control BRIN-BD11 cells, while the C20:3ω6/C18:3ω6 ratio was higher in the former than in the latter cells. A comparable situation prevails when comparing the liver phospholipid fatty acid pattern in control animals versus second-generation ω3-depleted rats [8]. Similarly, the lower C16:1ω7/C16:0 and C18:1ω9/C18:0 ratios found in the phospholipids of ω3-enriched cells, as compared with control BRIN-BD11 cells, duplicate the situation found in liver phospholipids when comparing control animals to second-generation ω3-depleted rats [8].

At variance with these findings, the prior exposure of the BRIN-BD11 cells to C20:5ω3 and C22:6ω3 not only augmented the triglyceride content of long-chain polyunsaturated ω3-fatty acids, but also that of several other fatty acids, the coinciding increase in the triglyceride content of the ω3-enriched cells contrasting with the finding that, in liver, such a total triglyceride content is lower in control animals than in second-generation ω3-deficient rats [8]. In other terms, both the supply of exogenous C20:5ω3 and C22:6ω3 to BRIN-BD11 cells and the dietary deprivation of long-chain polyunsaturated ω3-fatty acids in second-generation ω3-depleted rats resulted, somewhat paradoxically, in the accumulation of excessive amount of triglycerides in the BRIN-BD11 cells and in liver steatosis, respectively.

The metabolic data confirm that the tumoral BRIN-BD11 cells display a typical Crabtree effect [12]. The situation found when comparing the ω3-enriched cells to the control ones, differed vastly from that previously encountered when comparing the catabolism of D-glucose in islets obtained from normal animals versus second-generation ω3-depleted rats. In the latter case, the two major anomalies found in the latter rats consisted in a lesser increase in the generation of glucose metabolites in response to a given rise in hexose concentration and a lower ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization [2]. These two anomalies were tentatively attributed to inhibition of glucokinase by endogenous long-chain fatty acyl-coenzyme A [1]. In these experiments, however, the increase in either D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation in response to a rise in D-glucose concentration from 1.1 to 11.1 mM was less pronounced in the ω3-enriched cells than in the control cells. Moreover, no significant difference between control and ω3-enriched cells was observed in terms of the ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization, the trend being toward a lower ratio in ω3-enriched cells than in the control cells. In other words, the ω3-enriched BRIN-BD11 cells behaved in a manner reminiscent of that previously found in pancreatic islets prepared from ω3-depleted rats. It is tempting, therefore, to speculate that, in ω3-enriched BRIN-BD11 cells, the phosphorylation of D-glucose was altered in a manner comparable to that thought to prevail in pancreatic islet cells from ω3-depleted rats, possibly because of the considerable increase in the triglyceride and presumably fatty acid content of these ω3-enriched BRIN-BD11 cells.

In the control BRIN-BD11 cells, ouabain decreased, as expected, the net uptake of $^{86}Rb^{+}$. In these cells, such an uptake was, also as expected, higher in the presence of 1.1 mM D-glucose than in its absence. A further rise in hexose concentration to 11.1 mM severely decreased, however, $^{86}Rb^{+}$ net uptake. This coincides with the fact that the ATP content of BRIN-BD11 cells is also lower at high values than at low values of D-glucose concentration [13]. The ω3-enriched cells failed to differ significantly from the control cells in terms of the ouabain-induced decrease in $^{86}Rb^{+}$ net uptake. This again contrasts with the situation found when comparing islets from normal rats to those of ω3-depleted animals, in which case the ouabain-sensitive $^{86}Rb^{+}$ net uptake is much lower in the latter animals than in control rats [5]. Such a defect is corrected when ω3-deficient rats are injected intravenously 60–120 min before sacrifice with 1.0 ml of a medium-chain triglyceride:fish oil emulsion, as distinct from a medium-chain triglyceride:olive oil emulsion [5, 8]. The ω3-enriched cells differed, however, from the control cells in terms of their responsiveness to D-glucose. Indeed, in the ω3-enriched cells, the net uptake of $^{86}Rb^{+}$ failed to differ significantly in the absence or presence of 1.1 mM D-glucose, and was less severely decreased by a further rise in hexose concentration to 11.1 mM than in the control cells. The latter difference coincides with the lesser relative increase in D-glucose catabolism found in ω3-enriched cells, as distinct from control cells, in response to the same rise in D-glucose concentration from 1.1 to 11.1 mM.

Last, the comparison between the insulin secretory behavior of ω3-enriched cells versus control cells also differed from that otherwise found in pancreatic islets from normal rats versus ω3-depleted animals. Islets from ω3-depleted rats display an increased responsiveness to various nutrient and non-nutrient secretagogues, this situation being again opposed by the intravenous injection of the medium-chain triglyceride:fish oil emulsion 120 min before sacrifice [2, 4]. The ω3-enriched cells failed, however, to differ from control cells in terms of their insulin secretory responsiveness to D-glucose and theophylline. The sole difference between ω3-enriched and control BRIN-BD11 cells consisted in a lower absolute value for insulin output in the former as compared to latter cells. Such a decrease could not be attributed to any significant difference in the insulin content of control and ω3-enriched cells.

Taken as a whole, the present results reveal that a prior exposure for 24 h of BRIN-BD11 cells to C20:5ω3 and C22:6ω3 provokes change in metabolic, ionic, and secretory variables compatible with the concept of lipotoxicity and including cell steatosis, impaired D-glucose catabolism, lesser sensitivity to changes in extracellular D-glucose concentration in terms of $^{86}Rb^{+}$ net uptake, and lower absolute values for insulin release. In these several respects, the exogenous supply of long-chain polyunsaturated ω3-fatty acids to the BRIN-BD11 cells provoked metabolic and functional changes similar, rather than opposite, to those otherwise found when comparing islet cells from ω3-depleted rats to those from control animals. In considering such an unexpected analogy, it should be stressed that the relative ω3 fatty acid content of phospholipids in the control BRIN-BD11 cells was not lower than that found in islets from normal rats, averaging for the sum of C20:5ω3, C22:5ω3, and C22:6ω3 7.3 $\pm$ 0.5% ($n = 3$) in these BRIN-BD11 cells as compared ($P > 0.1$) to 5.9 $\pm$ 0.3% ($n = 2$) in pancreatic islets from normal rats [unpublished observation].

Materials and methods

BRIN-BD11 cells were grown and cultured as previously described [17]. During the last 24 h of culture, they were exposed, when so required, to albumin-bound C20:5ω3 and C22:6ω3 (50 μM each). Thereafter, distinct procedures were followed, depending on the variable to be measured.

For measuring the fatty acid content of cell phospholipids and triglycerides, the cells were washed twice with a Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (HBSS, Invitrogen, Merelbeke, Belgium) and detached after 20-min incubation and shaking at 37°C in 2.0 ml of the same Ca^{2+}- and Mg^{2+}-free HBSS. An aliquot part (0.5 ml) of the final cell suspension (20.0 ml) was mixed with an equal volume of H_2O, sonicated (3 times 10 s) and stored at −20°C for later measurement of protein [18] and insulin [17] content. A cell pellet was then obtained from the remaining cell suspension by centrifugation for 5 min at 1000*g*. The cell lipids were eventually extracted [19], separated by thin-layer chromatography [20], and their fatty acid pattern determined by gas–liquid chromatography [21].

The generation of ^{3}HOH from D-[5-^{3}H]glucose and that of $^{14}CO_2$ from D-[U-^{14}C]glucose were measured as previously described [22] over 90 min incubation at 37°C of the cells in 0.1 ml of a Hepes- and bicarbonate-buffered salt-balanced medium [23] containing 1.0 mg/ml bovine serum albumin, and equilibrated against a mixture of O_2:CO_2 (95:5, v:v).

The net uptake of $^{86}Rb^{+}$ was measured over 60 min incubation at 37°C in 0.15 ml of the same Hepes- and bicarbonate-buffered medium, the incubation medium being eventually separated from the cell pellet after addition of 0.15 ml of a dibutylphthalate/di-isononylphthalate oil mixture (10:3, v:v) by centrifugation for 3 min at 1000*g* [24].

Insulin release from attached cells was measured over 60 min incubation at 37°C [17].

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

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