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ORIGINAL ARTICLE

Fatty acid pattern of pancreatic islet lipids in Goto-Kakizaki rats

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Abstract Perturbations of fatty acid content and pattern were recently documented in epididymal and parametrial lipids, as well as plasma, liver, spleen, and brain phospholipids and triglycerides of Goto-Kakizaki rats (GK). This study extends such an investigation to pancreatic islets from both control and GK rats. Groups of 5,500–14,560 islets were obtained from either control or GK rats ($n = 3$ in each case) and examined for their lipid fatty acid content. In the islet triglycerides, the major difference between control and GK rats, i.e., a higher C18:2ω6 content in GK rats, was similar to that found in liver triglycerides. In the islet phospholipids, however, a number of differences between control and GK rats, concerning saturated, monodesaturated, and long-chain polyunsaturated ω3 and ω6 fatty acids, were often not similar to those found in liver phospholipids. The present study reveals a number of anomalies in the fatty acid pattern of islet phospholipids in GK rats, often differing from those encountered in liver phospholipids. Such a tissue specificity was borne out by the finding that, even in control animals, the situation found in islet phospholipids differed from that recorded in liver phospholipids.

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Introduction

Recent reports have drawn attention to the perturbation of the fatty acid content and pattern in epididymal or parametrial adipose tissue lipids, as well as plasma, liver, spleen, and brain phospholipids and triglycerides in animals models of both type 1 and type 2 diabetes [1–7]. To our knowledge, however, the fatty acid pattern of phospholipids and of triglycerides was so far never examined separately in each of these two lipid fractions in pancreatic islets of either normal or diabetic rats. The present report provides such information, as assessed in pancreatic islets isolated from control rats and Goto-Kakizaki rats, a current animal model of inherited type 2 diabetes.

Results

Age, body weight, plasma glucose, and insulin concentration

Table 1 provides information on the age, body weight, plasma D-glucose, and insulin concentrations, as well as plasma insulin/glucose ratio, in the 72 control fed male rats and 116 fed male GK rats used to isolate a total number of 50,380 islets and to eventually prepare six separate batches of islets for lipid analysis. Pooling all available data, the age of the control animals (13.5 ± 0.4 weeks; $n = 72$) and GK rats (14.2 ± 0.2 weeks; $n = 116$) failed to differ significantly ($P > 0.05$) from one another. The body weight was much lower ($P < 0.001$), however, in GK rats

Table 1 Age, body weight, and plasma glucose, and insulin concentrations

Samples number	1.		2.		3.	
Collection period	December 2005 to January 2006		August 2005 to April 2007		February 2008 to June 2008	
Rats	Control	GK	Control	GK	Control	GK
Age (weeks)	14.4 ± 0.5 (17)	13.5 ± 0.4 (26)	16.4 ± 0.4 (28)	16.0 ± 0.3 (48)	10.0 ± 0.2 (27)	12.5 ± 0.2 (42)[a]
Body weight (g)	451 ± 17 (17)	287 ± 4 (26)[a]	449 ± 11 (19)	307 ± 7 (22)[a]	386 ± 11 (24)	285 ± 5 (42)[a]
Plasma glucose (mM)	7.90 ± 0.48 (14)	12.99 ± 0.74 (26)[a]	7.63 ± 0.16 (19)	12.10 ± 0.61 (30)[a]	7.13 ± 0.13 (26)	10.51 ± 0.50 (42)[a]
Plasma insulin (ng/ml)	3.06 ± 0.24 (14)	2.83 ± 0.20 (26)	2.44 ± 0.12 (19)	3.39 ± 0.13 (30)[a]	3.08 ± 0.20 (26)	4.10 ± 0.13 (42)[a]
Insulin/glucose (μg/mol)	401 ± 38 (14)	231 ± 22 (26)[a]	324 ± 20 (19)	303 ± 23 (30)	431 ± 27 (26)	439 ± 29 (42)
Islet number	6,050	6,120	8,510	6,500	12,100	11,100

[a] $P < 0.001$ (GK versus control rats)

(291 ± 3 g; $n = 90$) than in control animals (424 ± 11 g; $n = 60$). The plasma D-glucose concentration was higher ($P < 0.001$) in GK rats (11.65 ± 0.36 mM; $n = 98$) than in control animals (7.47 ± 0.14 mM; $n = 59$). Even the plasma insulin concentration was significantly higher ($P < 0.001$) in GK rats (3.54 ± 0.10 ng/ml; $n = 98$) than in control animals (2.87 ± 0.12 ng/ml; $n = 59$). The plasma insulin/glucose ratio failed to differ significantly ($P > 0.1$), however, in GK rats (342 ± 20 μg/mol; $n = 98$) and control animals (389 ± 17 μg/mol; $n = 59$), a significant decrease ($P < 0.001$) of such a ratio in GK rats being only observed in the animals used to prepare the first islet samples.

Samples 1 and 2 were pooled together prior to lipid analysis, which was conducted on 14, 560 islets from control animals and 12,620 islets from GK rats. In these pooled samples, the fatty acid patterns of triglycerides and phospholipids were established after separation of these two classes of lipids. In the case of samples 3, however, the fatty acid pattern of islet lipids was measured without prior separation of phospholipids and triglycerides. Moreover, samples 3 allowed two separate determinations in both control animals (6,100 and 6,000 islets) and GK rats (5,500 and 5,600 islets).

Triglycerides

The total fatty acid content of islet lipids failed to differ significantly ($P > 0.6$) in control animals (25.4 ± 1.5 mg/g wet wt; $n = 3$) and GK rats (26.9 ± 2.2 mg/g wet wt; $n = 3$). Relative to the total fatty acid content of islet lipids, that of triglycerides did not exceed 3.67 ± 0.63‰ ($n = 2$).

In the islet triglycerides, the most abundant fatty acids were C16:0, C18:1ω9, C18:2ω6, C20:4ω6, C18:0, and C20:3ω6. Their relative weight content (%) averaged ($n = 2$ in all cases) 35.0 ± 1.5 for C16:0, 22.6 ± 1.0 for C18:1ω9, 13.3 ± 3.7 for C18:2ω6, 12.3 ± 0.4 for C20:4ω6, 5.2 ± 2.3 for C18:0, and 2.3 ± 0.3 for C20:3ω6. There was a highly significant positive correlation ($r = 0.7663$; $n = 12$; $P < 0.005$) between the relative weight content of these six fatty acids in the islet and liver triglycerides of control and GK rats [1].

In the case of islet triglycerides, the most striking difference between control animals and GK rats consisted in a higher C18:2ω6 relative weight content in the GK rat islets (16.9%) than in the control rat islets (9.6%). This finding is reminiscent of that recorded in liver triglycerides [1] in which case such a C18:2ω6 relative weight content is also higher ($P < 0.005$) in GK rats (35.8 ± 1.0%; $n = 4$) than in control animals (22.9 ± 2.1; $n = 4$). In this respect, the GK/control ratio for the relative C18:2ω6 content of triglycerides was closely comparable in islets (1.76) and liver (1.56).

Whilst the (C18:0 + C18:1ω9)/(C16:0 + C16:1ω7) ratio (0.790 ± 0.060; $n = 2$) and C20:4ω6/C20:3ω6 ratio (5.48 ± 0.46; $n = 2$) were not vastly different in control and GK rats, both the C20:3ω6/C18:2ω6 and C20:4ω6/C18:2ω6 were much lower in GK rats (0.119 and 0.71) than in control animals (0.263 and 1.32). The C18:1ω9/C18:0 ratio was also about twice lower in GK rats (3.18) than in control animals (7.49).

Phospholipid saturated fatty acids

The mean relative weight content of C12:0, C14:0, and C16:0 in islet phospholipids was higher in GK rats than in control animals (Table 2). As judged from the GK/control ratio (Fig. 1), this difference achieved statistical significance (109.5 ± 1.6%; df = 4; $P < 0.005$) in the case of C16:0, but not so in the case of C14:0 (111.8 ± 5.1%; df = 4; $P < 0.1$). The total amount of C12:0, C14:0, and C16:0 also yielded a GK/control ratio significantly higher than unity (109.7 ± 1.7%; df = 4; $P < 0.005$).

Table 2 Relative weight content (‰) of fatty acids in islet phospholipids

Fatty acid	Control rats	GK rats
C12:0	0.17 ± 0.17 (3)	0.50 ± 0.29 (3)
C14:0	2.09 ± 0.07 (3)	2.34 ± 0.07 (3)
C16:0	199.5 ± 10.2 (3)	218.2 ± 8.2 (3)
C18:0	164.9 ± 6.1 (3)	159.7 ± 2.8 (3)
C20:0	2.43 ± 0.32 (3)	1.17 ± 0.58 (3)
C22:0	2.70 ± 0.07 (3)	2.71 ± 0.07 (3)
C24:0	8.00 ± 0.13 (3)	8.42 ± 0.33 (3)
C16:1ω7	3.12 ± 0.61 (3)	4.25 ± 0.80 (3)
C18:1ω9	50.54 ± 1.60 (3)	47.55 ± 1.32 (3)
C20:1ω9	0.70 ± 0.70 (3)	1.35 ± 0.68 (3)
C22:1ω9	4.96 ± 1.93 (3)	1.39 ± 0.99 (3)
C18:2ω6	126.0 ± 5.5 (3)	106.6 ± 2.7 (3)[a]
C18:3ω6	1.85 ± 0.17 (3)	2.71 ± 0.05 (3)[c]
C20:2ω6	6.22 ± 0.25 (3)	4.43 ± 0.07 (3)[d]
C20:3ω6	19.87 ± 2.16 (3)	19.58 ± 1.64 (3)
C20:4ω6	331.4 ± 4.1 (3)	338.7 ± 2.3 (3)
C22:4ω6	12.48 ± 0.89 (3)	13.14 ± 1.04 (3)
C18:3ω3	0.41 ± 0.41 (3)	0.31 ± 0.31 (3)
C18:4ω3	0 ± 0 (3)	0 ± 0 (3)
C20:5ω3	6.75 ± 0.70 (3)	6.85 ± 0.01 (2)
C22:5ω3	10.68 ± 1.20 (3)	13.82 ± 1.62 (3)
C22:6ω3	37.83 ± 1.3 (3)	48.36 ± 2.17 (3)[b]

[a] $P < 0.05$; [b] $P < 0.02$, [c] $P < 0.01$, and [d] $P < 0.005$

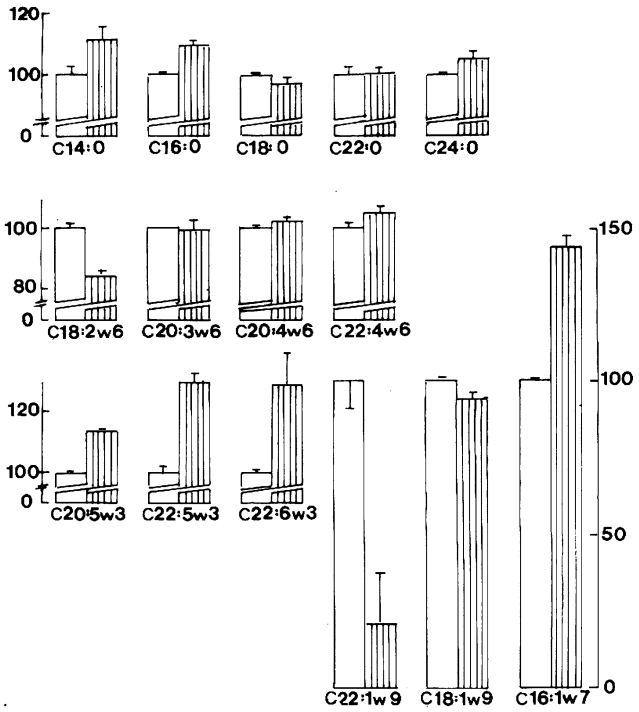

Fig. 1 Mean values (± SEM) for the phospholipid relative weight content of fatty acids in islets from control (*open columns*) and GK (*hatched columns*) rats. All results are expressed by reference to the mean control value(s) found within each set of measurements and refer to 2–3 separate determinations

No significant increase in the phospholipid relative content of further saturated fatty acids (C18:0, C20:0, C22:0, and C24:0) was anymore observed in the GK rats. The total amount of these four fatty acids indeed yielded a GK/control ratio averaging 96.8 ± 2.3% (df = 4; $P > 0.2$). Likewise, a GK/control overall ratio averaging 96.2 ± 3.6% (df = 21; $P > 0.3$) was reached when pooling together the results obtained for the GK/control ratio of each of these four fatty acids.

As expected from these findings, the C18:0/C16:0 ratio was lower in GK rats than in control animals (Table 3), yielding a GK/control percentage (88.6 ± 3.0%; df = 4; $P < 0.02$) significantly lower than unity (Fig. 2). Likewise, the (C18:0 + C18:1ω9)/(C16:0 + C16:1ω7) ratio also yielded a GK/control percentage lower than unity (87.6 ± 2.8%; df = 4; $P < 0.02$), suggesting decrease activity of elongase in the diabetic rats.

Phospholipid monodesaturated fatty acids

Among the four monodesaturated fatty acids examined in this study, only C16:1ω7 displayed a higher relative weight content in the islet phospholipids of GK rats, as compared to control animals. It yielded a GK/control ratio averaging 143.9 ± 3.6% (df = 4; $P < 0.001$ versus unity). In the case of C18:1ω9, the GK/control ratio (94.2 ± 2.0%; df = 4) was significantly lower than unity ($P < 0.05$).

The C16:1ω7/C16:0 ratio also yielded a GK/control percentage (131.4 ± 1.8%; df = 4) higher than unity ($P < 0.001$), suggesting increased activity of Δ9-desaturase (Fig. 2). In the case of the C18:1ω9/C18:0 ratio, however, the values recorded in the control animals (306 ± 4‰; $n = 3$) and in the GK rats (298 ± 6‰; $n = 3$) were closely comparable to one another ($P > 0.25$).

No significant difference between control and GK rats was observed concerning the C20:1ω9 relative weight content, whether null values recorded in three out of six rats were taken into account (Table 2) or not, in which case such a relative weight content represented 2.10‰ in a control animal and 2.03 ± 0.14‰ in two GK rats. The mean C22:1ω9 relative weight content was lower, however, in GK rats than in control animals (Table 2), yielding a GK/control ratio of 24.4 ± 44.9% ($P < 0.2$). As illustrated in Fig. 1, when excluding one abnormally low value (1.17‰), well below the other readings recorded in control rats (6.86 ± 0.92‰), the GK/control ratio amounted to 21.6 ± 22.0% (df = 3; $P < 0.05$). Whenever both C20:1ω9 and C22:1ω9 could be detected in the islet phospholipids, the C22:1ω9/C20:1ω9 ratio amounted to

Table 3 Ratio between selected fatty acids in islet phospholipids

Fatty acids	Control rats	GK rats
C18:0/C16:0 (‰)	834 ± 69 (3)	735 ± 39 (3)
(C18:0 + C18:1ω9)/(C16:0 + C16:1ω7)	1.07 ± 0.08 (3)	0.93 ± 0.05 (3)
C16:1ω7/C16:0 (‰)	15.0 ± 3.1 (3)	19.8 ± 4.2 (3)
C18:1ω9/C18:0 (‰)	306 ± 4 (3)	298 ± 6 (3)
C22:1ω9/C20:1ω9	2.96 (1)	1.07 ± 0.67 (2)
C20:2ω6/C18:2ω6 (‰)	49.7 ± 4.0 (3)	41.6 ± 1.6 (3)
C22:4ω6/C18:2ω6 (‰)	100 ± 11 (3)	124 ± 13 (3)
C22:6ω3/C20:5ω3	5.69 ± 0.40 (3)	7.36 ± 0.20 (2)
C22:5ω3/C22:6ω3 (‰)	284 ± 38 (3)	284 ± 22 (3)

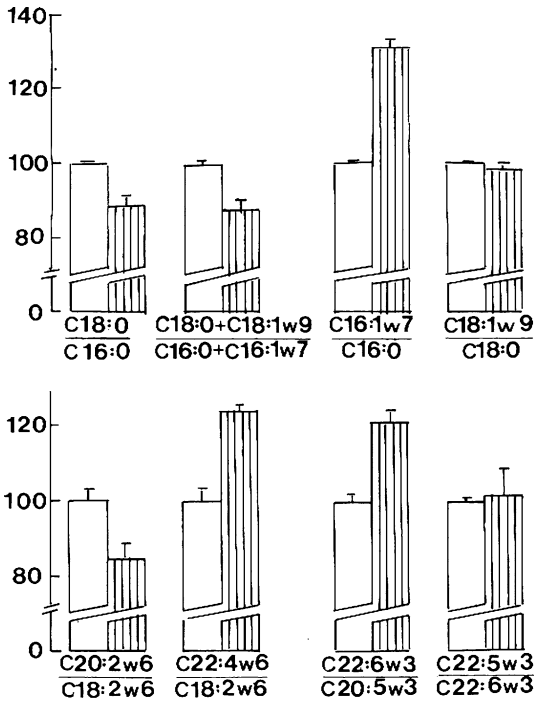

Fig. 2 Mean values (± SEM) for the ratio between selected fatty acids in islet phospholipids. Same presentation as in Fig. 1

2.96 ($n = 1$) and 1.07 ± 0.67 ($n = 2$) in control and GK rats, respectively. These findings are compatible with a decreased net generation, in the GK rats, of C22:1ω9 in the pathway leading to the formation of nervonic acid (C24:1ω9).

Phospholipid long-chain polyunsaturated ω6 fatty acids

The C18:2ω6 and C20:2ω6 contents of islet phospholipids were both significantly lower in GK rats than in control animals (Table 2). Even the C20:2ω6/C18:2ω6 paired ratios yielded a mean GK/control percentage not exceeding 84.4 ± 5.0% (df = 4; $P < 0.05$ versus unity). A different situation prevailed for the stepwise conversion of C18:2ω6 to C22:4ω6. Indeed, whilst the GK/control ratio for the relative content of C18:2ω6 in islet phospholipids averaged 84.7 ± 1.9% (df = 4; $P < 0.005$ versus unity) it progressively increased, as illustrated in Fig. 1, to 99.2 ± 3.2% in the case of C20:3ω6 and 102.2 ± 1.1% in the case of C20:4ω6, to eventually reach 105.1 ± 2.2% in the case of C22:4ω6 (df = 4, in all cases). In other words, the C22:4ω6/C18:2ω6 paired ratio yielded a GK/control percentage of 124.0 ± 3.3% (df = 4; $P < 0.005$ versus unity), suggesting facilitated stepwise conversion of C18:2ω6 to C22:4ω6 in the diabetic rats (Fig. 2).

Phospholipid long-chain polyunsaturated ω3 fatty acids

As judged from the data listed in Table 2, the sole difference between control and GK rats, in terms of long-chain polyunsaturated ω3 fatty acid relative content of islet phospholipids, consisted in an increased C22:6ω3 content ($P < 0.02$) in the diabetic animals. However, the C20:5ω3, C22:5ω3, and C22:6ω3 content of islet phospholipids yielded GK/control ratios significantly exceeding unity and averaging 113.3 ± 0.6% (df = 3; $P < 0.005$), 129.3 ± 3.5% (df = 4; $P < 0.001$), and 128.3 ± 9.0% (df = 4; $P < 0.05$). The C22:6ω3/C20:5ω3 ratio was also significantly higher ($P < 0.05$) in GK rats than in control animals (Fig. 2), whilst such was not the case for the C22:5ω3/C22:6ω3 ratio, which yielded a GK/control percentage of 101.7 ± 6.9% (df = 4; $P > 0.8$).

Comparison of islet and liver data

In the islet phospholipids of control rats, the weight content of fatty acids displayed the following hierarchy: C20:4ω6 > C16:0 > C18:0 > C18:2ω6 > C18:1ω9 > C22:6ω3 > C20:3ω6 > C22:4ω6 ≥ C22:5ω3, the other fatty acids representing each less than 1% of the total amount of phospholipid fatty acids. There was a highly significant positive correlation ($r = +0.9361$; df = 20; $P < 0.001$) between the relative weight content of the 22 fatty acids listed in Table 1 as recorded in the islet phospholipids of control rats and the corresponding values previously reported in the liver phospholipids of control animals (1). Nevertheless, significant differences between the relative weight content of certain fatty acid were encountered, when comparing islet and liver phospholipid data. For instance the C16:0 and C18:0 relative weight content averaged, respectively, 199 ± 10 and 165 ± 6‰ in islets as distinct ($P < 0.02$ or less) from 165 ± 4 and 240 ± 2‰ in the liver. Hence, the C18:0/C16:0 ratio was also vastly different ($P < 0.002$) in islets (0.83 ± 0.07) and liver (1.62 ± 0.05). Moreover, the C18:1ω9 relative weight content in islets (5.1 ± 0.2%) exceeding ($P < 0.001$) that

found in liver (2.8 ± 0.1%), the C18:1ω9/C18:0 ratio in islets (0.31 ± 0.01) was also higher ($P < 0.001$) than that found in liver (0.12 ± 0.01). Another striking example consisted in the lower C22:6ω3 relative weight content ($P < 0.001$) in islet phospholipids (3.8 ± 0.1%) than in liver phospholipids (12.8 ± 0.4%).

Likewise, the differences in the islet phospholipid pattern of fatty acids between control and GK rats were not uncommonly opposite to those previously documented in liver phospholipids [1, 2]. For instance, such was the case for the weight content of C14:0 and C16:0, and that of C18:2ω6, as well as for the C16:1ω7/C16:0 and C20:2ω6/C18:2ω6 ratios.

Discussion

In prior reports, several metabolic and functional anomalies were already documented in islets prepared from GK rats by the same technique as that used in the present study. Thus, emphasis was placed on a preferential alteration of oxidative glycolysis coinciding (i) with a deficiency of FAD-linked glycerophosphate dehydrogenase, the key enzyme of the glycerol phosphate shuttle, (ii) with a low ATP/ADP ratio in islets exposed to a high concentration of glucose, and (iii) with an impaired response of perifused islets to a rise in D-glucose concentration in terms of stimulation of insulin release, reduction of ^{86}Rb efflux, and induction of a phosphate flush [8–10].

Only scanty information was so far available on the fatty acid pattern of triglycerides and phospholipids in pancreatic islets, even in normal animals. Montague and Parkin first reported that, in groups of 200 isolated pancreatic islets from guinea pig incubated for 30 min at 37°C in the presence of 20 mM glucose and 0.5 mM isobutylmethylxanthine, there was a significant increase in C18:3ω6 and C20:3ω6 relative to the control values recorded when the islets were incubated for 30 min at a low glucose concentration (4 mM). It was proposed that these changes in unsaturated fatty acid content might be expected to produce an increase in the fluidity of the plasma membrane [11]. Shortly thereafter, Deleers et al. indeed documented that, within 1 min after increasing the extracellular glucose concentration from 0 to 5.6, 11.1, and 16.7 mM, the hexose provokes a concentration-related decrease in the fluorescence polarization of rat-dispersed pancreatic islet cells labelled with 1,6-diphenyl-1,3,5-hexatriene, indeed suggesting an increase in membrane fluidity [12]. At a glucose concentration of 16.7 mM, the estimated viscosity was 15 ± 3% lower than basal value, i.e., 2.01 ± 0.12 P. In considering environmental changes in the fatty acid pattern of islet lipids, mostly phospholipids, it should be kept in mind, however, that, taking into account the measurements made by Dean in mice islets [13], it may be calculated that the mean ß-cell surface (973 μ^2) represents no more than about 7% of the total surface of the plasma membrane, secretory granules, mitochondria, nucleus, and endoplasmic reticulum. Incidentally, in the case of the eight fatty acids (C16:0, C16:1ω7, C18:0, C18:1ω9, C18:2ω6, C18:3ω3, C20:3ω6, and C20:4ω6) measured both by Montague and Parkin [11] in guinea pig islets and in the present study in islets from control rats there was a significant correlation ($r = 0.7105$; $n = 8$; $P < 0.05$) between the two sets of results, which accounted for 91.7 ± 2.0% of the total amount of fatty acids.

Further information on the fatty acid pattern of islet lipids is summarized in Table 4, which documents the fair analogy between the results of two previous studies [14, 15] conducted in normal rats and those of the present one on a limited number of variables examined in all reports under consideration.

To our knowledge, only two pieces of information were so far available on the lipid content and fatty acid pattern of pancreatic islets from GK rats. Briaud et al. [16] first reported that the islet total triglyceride content failed to differ significantly in control Wistar rats (150 ± 43 ng/islet) and GK rats (194 ± 35 ng/islet). Expressed in the same manner, the presented results averaged 127 ± 8 ng/islet in control animals and 135 ± 11 ng/islet in GK rats, there being no significant difference ($P > 0.3$) between the mean values derived from these two separate studies. More recently, Nunes et al. [17] observed, in fair agreement with the present results, a 15% higher C16:1ω7 relative content in the total lipids of islets from female GK rats as compared to female Wistar rats of similar age. The same authors did not provide information, however, on the total fatty acid content of islet lipids and their relative content in the long-chain polyunsaturated ω3 fatty acids C20:5ω3, C22:5ω3, and C22:6ω3.

The present work provides extensive information on the fatty acid content and pattern of triglycerides and phospholipids in pancreatic islets isolated from normal rats, and reveals a number of differences between islets and liver in these respects. To cite only one example, the relative contribution of triglycerides to the total fatty acid content of islet lipids did not exceed 3.67 ± 0.63‰, as distinct from a value of 167.9 ± 14.3‰ in the liver of normal rats [1]. The fatty acid content of phospholipids was comparable, however, in pancreatic islets (25.4 ± 1.5 mg/g) and liver (25.2 ± 0.3 mg/g) of normal rats. The low ratio between the fatty acid content of triglyceride and phospholipid in the islet cells is reminiscent of the situation found in rat brain, in which case such a content averages 0.20 ± 0.04 mg/g in triglycerides and 33.6 ± 1.1 mg/g in phospholipids, yielding a triglyceride/phospholipid ratio close to 6.0‰ [7].

Table 4 Comparison of lipid data collected in normal rats in three distinct studies

Authors	Yazici et al. [21]	Oguzhan et al. [22]	Giroix et al.
Total fatty acid content (ng/islet)	117 ± 16 (5)	101 ± 12 (14)	127 ± 8 (5)
C18:0/C16:0 ratio	1.08 ± 0.06 (5)	0.96 ± 0.20 (2)	0.83 ± 0.07 (3)
C18:1ω9/C18:0 ratio	0.62 ± 0.04 (5)	0.36 ± 0.04 (2)	0.31 ± 0.01 (3)
C20:4ω6/C18:2ω6 ratio	1.50 ± 0.07 (5)	2.11 ± 0.33 (3)	2.64 ± 0.14 (3)
C20:5ω3 + C22:5ω3 relative content (%)	ND	1.15 ± 0.14 (2)	1.74 ± 0.05 (3)

ND not determined

No obvious increase in the triglyceride total fatty acid content was observed in the islets of GK rats. Likewise, the fatty acid content of triglycerides in the liver of female fed rats is not higher in GK rats (3.61 ± 0.52 mg/g) than in normal animals (5.14 ± 0.47 mg/g). An increased relative weight content of C18:2ω6 in the islet triglycerides of GK rats was also in fair agreement with previously reported liver data [1].

At variance with the latter findings, the fatty acid pattern of phospholipids not only differed, in control animals, between pancreatic islets and liver, but also the alteration of such a pattern in the pancreatic islets of GK rats, as compared to control animals, was also often different from those previously reported when comparing the situation found in the liver phospholipids of GK rats versus control animals. As a matter of fact, such dual differences were already documented in prior reports dealing with the fatty acid pattern of phospholipids in other organs, such as spleen and brain of control and GK rats [6, 7].

The findings in the islet phospholipids suggest a decreased activity of elongase in the GK rats with low C18:0/C16:0, (C18:0 + C18:1ω9)/(C16:0 + C16:1ω7), C20:2ω6/C18:2ω6, and C22:1ω9/C20:1ω9 ratios, an increase activity of Δ9-desaturase with a high C16:1ω7/C16:0 (but not C18:1ω9/C18:0) ratio, and facilitated stepwise conversion of both C18:2ω6 to C22:4ω6 and C20:5ω3 to C22:6ω3.

The present findings do not allow to decide whether the observed differences between the fatty acid pattern of phospholipids in GK rats versus control animals represent inherited or secondary features. In this respect, it merits to be underlined that there was a close analogy between the changes in the fatty acid pattern of islet lipids observed by Montague and Parkin [11] when raising the extracellular concentration of D-glucose and the differences recorded in the present study when comparing GK to control rats. In the first instance, both the C18:3ω6/C18:2ω6 and C20:3ω6/C18:2ω6 were significantly increased ($P < 0.001$), respectively from 53.1 ± 9.7‰ to 191.5 ± 21.3‰ and from 82.1 ± 9.7‰ to 180.6 ± 20.8‰ ($n = 8$ in all cases), the former increase being, in relative terms, more marked ($P < 0.02$) than the latter one. Likewise, the GK/control percentages for both the C18:3ω6/C18:2ω6 and C20:3ω6/C18:2ω6 ratios significantly exceeded unity ($P < 0.05$), averaging, respectively, 176.5 ± 21.7 and 117.2 ± 5.9% (df = 4 in both cases), the former percentage also exceeding ($P < 0.04$) the latter one. Hence, it could be argued that the differences between GK and control rats, in terms of the C18:3ω6/C18:2ω6 and C20:3ω6/C18:2ω6 ratios, merely reflect the higher extracellular glucose concentration found in GK rats, as distinct from control animals.

In conclusion, the present study reveals several differences between GK and control rats in the fatty acid pattern of pancreatic islet phospholipids. Such differences may well participate in the fine regulation of metabolic and functional events in the islet cells of these animals, for instance through changes in membrane fluidity and/or by modulating the activity of enzymes, e.g., Na^+, K^+-ATPase [18, 19], known to be affected in their catalytic properties by changes in their phospholipid environment. It is also conceivable that the altered fatty acid pattern of islet phospholipids in GK rats is somehow involved in the perturbation of islet phosphoinositide metabolism previously documented in this rat model of type 2 diabetes [20].

Materials and methods

Animals

Animal experimentation was performed in accordance with accepted standards of animal care as established in the French National Center for Scientific Research guidelines. GK rats were obtained from our local colony at University Paris-Diderot which was initiated in 1988 [21] with progenitors issues from the 34th generation in the original colony established by Goto and Kakizaki [22]. Wistar rats raised in parallel were used as control animals. The rats, all male adult, were given free access to tap water and a standard pelleted chow (diet 113; Usine d'Alimentation Rationnelle, Villemoison-sur-Orge, France) up to the time of the experiments. This diet contained (g/kg) 6.7 C16:0, 0.5 C16:1ω7, 2.7 C18:0, 14.9 C18:1ω9, 19.7 C18:2ω6, and trace amounts of C18:3ω3.

Blood collection and preparation of pancreatic islets

On the morning (9–11 a.m.) the rats were weighed and sacrificed by decapitation, blood being collected in

heparinized tubes. After centrifugation the plasma was removed and stored at −20°C for further determination of plasma glucose by the glucose oxidase method [23] using glucose analyzer (Beckman instruments) and plasma insulin by radioimmunoassay [24]. In each experiment, islets were isolated from the pancreas(es) of 1–5 rats in a given group (control or GK rats) using a collagenase (EC 3.4.24.3 from *Clostridium histolyticum*, type P; Roche Molecular Biochemicals, Mannheim, Germany) digestion procedure derived from a method previously described [25] and passed through a 400 mesh nylon filter (Nytal, Thal, Switzerland) to discard large exocrine aggregates. The islets were further isolated by two consecutive collections in an ice-cold bicarbonate- and HEPES-buffered salt-balanced medium [26] supplemented with fatty acid-free bovine serum albumin (BSA, 5 mg/ml) and D-glucose (5.6 mM) by hand-picking under control of a stereomicroscope. After centrifugation the collect medium was removed and the islets (by batches of 100–700 each) were washed two times with 2 ml Hank's balanced salt solution without BSA and glucose. The washing medium was removed, the islets immediately frozen in liquid N_2 and then stored at −80°C until analysis of their lipid fatty acid composition.

Lipid analysis

The cell lipids were extracted [27] and, when so required, separated by thin-layer chromatography [28]. The fatty acid pattern of cell lipids was determined by gas liquid chromatography [29].

Presentation of results

All results are expressed as mean values ± SEM, together with the number of separate determinations (*n*) or degree of freedom (df). Absolute values for the variables under consideration are listed in Tables 1, 2, 3. They were established on the basis of a mean wet weight of 5.0 µg per islet [30]. The GK/control ratios for the same variables were calculated by reference to the mean control value(s) found within each set of measurements (Figs. 1 and 2). In the text, the SEM on such GK/control ratios takes into account the dispersion of results in both the control and GK rats. The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

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