Metabolism of endogenous nutrients in islets of Goto–Kakizaki (GK) rats

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The metabolism of endogenous nutrients was examined in pancreatic islets of control and Goto–Kakizaki (GK) rats. At the ultrastructural level, no glycogen was found in the islet cells of GK rats, a situation similar to that prevailing in normal islets. Likewise, by measuring the output of L-lactate from islets first incubated at 16.7 mM D-glucose and then at 2.8 mM D-glucose, no evidence of glycogenolysis was found in the islets of GK rats. The production of NH_4^+ and that of $^{14}\text{CO}_2$ from islets prelabelled with either L-[U- ^{14}C]glutamine or [U- ^{14}C]palmitate were higher, however, in GK than in control rats. The changes in NH_4^+ and $^{14}\text{CO}_2$ production evoked by D-glucose, by a non-metabolized analogue of L-leucine (2-amino-bicyclo[2,2,1]heptane-2-

carboxylic acid; BCH) and by 3-phenylpyruvate were qualitatively comparable in control and GK rats. The secretory response to these three secretagogues was severely decreased in the islets of GK rats. This coincided with an impaired enhancing action of D-glucose on the conversion of [2- ^3H]glycerol into ^3HOH . It is concluded that the catabolism of endogenous amino and fatty acids in islets is greater in GK than in control rats, especially at low D-glucose concentration. This may account, in part at least, for the altered secretory response to BCH and 3-phenylpyruvate. For glucose-induced insulin release, however, an impaired acceleration of the glycerol phosphate shuttle apparently also participates in the secretory defect.

INTRODUCTION

In Goto–Kakizaki (GK) rats, non-insulin-dependent diabetes represents an inherited disease [1,2]. It was recently proposed that the alteration of glucose-induced insulin release in GK rats [3–5] may be due, in part at least, to a deficiency of FAD-linked glycerophosphate dehydrogenase in the pancreatic B-cell [6,7]. Our previous investigations also led us to speculate that the utilization rate of endogenous nutrients might be higher in islets of GK than of control rats, especially when the islets are exposed to a low concentration of D-glucose [8]. Such a view is based on three independent series of findings. First, at 2.8 mM D-glucose, the ATP/ADP ratio tends to be higher in islets of GK than of control rats [8]. Second, at the same low hexose concentration, the fractional outflow rate of ^{86}Rb from prelabelled islets is lower in GK than control rats [8]. Last, in islets exposed to 5.1 mM D-glucose, the ratio between D-[6- ^{14}C]glucose and D-[2- ^{14}C]glucose oxidation is also much lower in GK than in control rats [6]. In the light of these findings, the major aim of the present study was to investigate precisely the catabolism of endogenous nutrients in islets of GK rats.

MATERIALS AND METHODS

In this work, 24 male 3-month-old diabetic GK rats were examined together with 24 male age-matched control rats of the same strain. All animals were bred at the Karolinska Institutet (Stockholm, Sweden), shipped to Brussels (Belgium), housed (3–4 rats per cage) in a thermostatically controlled room (22 °C) with a 12 h-light/12 h-dark cycle, and given free access to water and food (AO4; Animalabo, Villemoisson s/Orge, France) until killed at 08:00–09:00h, 6–14 days later.

The D-glucose [9] and insulin [10] concentrations were measured in the plasma separated from blood collected at the time of death in heparinized tubes.

The methods used to isolate pancreatic islets [11] and to measure their protein [12] and insulin [11] contents, the total output of L-lactic acid and that of L-[^{14}C]lactic acid [13], the production of NH_4^+ [14], the conversion of [2- ^3H]glycerol into ^3HOH and tritiated acidic metabolites [15], the output of $^{14}\text{CO}_2$ from islets prelabelled with either L-[U- ^{14}C]glutamine [16] or [U- ^{14}C]palmitate [17], and the release of insulin [12], were previously described in the cited references. In the experiments designed to measure the output of L-lactic acid, the Hanks solution used for isolation of the islets contained 16.7 mM D-glucose for both control and GK rats. In some of these experiments, the output of both ^{14}C -labelled and total L-lactic acid was measured in each group of islets, allowing establishment of a paired ratio between the two measurements. Metabolic and secretory data were often expressed relative to the mean protein content of islets collected from the same initial batch.

For morphological examination, in two separate experiments, two groups of 20 islets isolated from control rats and GK rats, respectively, were fixed in 2.5% (v/v) glutaraldehyde in cacodylate buffer (0.1 M, pH 7.4). After several rinses, and postfixation in OsO_4 (4%, v/v) in the same buffer, the islets were dehydrated in graded solutions of ethanol, and eventually embedded in Epon. Semi-thin sections (1 μm) were examined by phase-contrast microscopy. Ultrathin sections, counterstained with uranyl acetate and lead citrate, were examined in a Philips 301 electron microscope. For each type of animal, a total of 8–10 islets were examined by phase-contrast microscopy, from which 5 selected islets were examined at the ultrastructural level.

All results are presented as mean values ($\pm$ S.E.M.) together with either the number of individual observations (n), each of which was performed in a separate group of islets, or the degree of freedom (d.f.). The statistical significance of differences between mean values was assessed by use of Student's unpaired t test.

RESULTS

Metabolic status of control and GK rats

Although of the same age, the GK rats displayed a lower body weight ($P < 0.001$) than control animals (Table 1). In the fed state, both the plasma D-glucose and insulin concentrations were higher ($P < 0.005$ or less) in GK than in control rats. The protein content of the islets collected in the present study was slightly, but not significantly ($P > 0.06$), higher in GK than in control rats. The insulin content of the islets was markedly decreased ($P < 0.04$). We have previously documented that the relative contribution of B-cells to total islet mass is not altered in the GK rats [7].

Morphological findings

In the islets of GK rats examined by phase-contrast microscopy, several B-cells appeared less granulated than others and displayed perinuclear vacuolization, either circular or crescent-shaped (Figure 1). At the ultrastructural level, the vacuoles were found to correspond to a dilation of the elements of the Golgi apparatus. In the same cells, cisternae of the rough endoplasmic reticulum appeared prominent. No glycogen deposit was detected, in either normal or GK-rat islets.

L-Lactate production by islets

The first series of experiments was aimed at exploring whether glycogenolysis takes place in islets prepared from GK rats and incubated at a low concentration of D-glucose. Two findings argue against such an idea.

First, and as mentioned above, no glycogen was found by ultrastructural examination in the B-cells of GK rats.

Second, when islets were incubated for two successive periods of 10 min each, first at 16.7 mM D-glucose and then at 2.8 mM D-glucose, the output of L-lactic acid from islets of either control or GK rats decreased to the same relative extent, i.e. to 50% (Table 2). This situation sharply contrasts with that found in glycogen-rich islets, in which no decrease in L-lactic acid output occurs when the concentration of D-glucose is decreased from 16.7 mM during the first incubation to 2.8 mM D-glucose during the second incubation [13]. In the present experiments, the absolute values for L-lactic acid output were slightly, but not significantly, lower in GK than in control rats.

In some of the present experiments, D-[U- ^{14}C]glucose was present in the incubation medium. The fall in hexose con-

centration also decreased the output of L-[^{14}C]lactic acid in both control and GK rats. The absolute values for the generation of L-[^{14}C]lactic acid were virtually identical in control and GK rats.

As expected from the presence of a rather large pool of unlabelled L-lactic acid inside the islet cells at the onset of the first incubation [18], the specific radioactivity of L-lactate released by the islets in the extracellular medium was lower than that of exogenous D-glucose, in fair agreement with a previous observation [13]. As judged from paired measurements of both ^{14}C -labelled and unlabelled L-lactic acid, the specific radioactivity of this extracellular metabolite averaged, in control and GK rats respectively, $50.5 \pm 4.7\%$ and $47.7 \pm 5.9\%$ during the first incubation and $45.6 \pm 10.5\%$ and $42.4 \pm 6.6\%$ during the second incubation ($n = 5$ in all cases), all values being expressed relative to the specific radioactivity of exogenous D-glucose. The trend towards a fall in L-lactate specific radioactivity during the second incubation may reflect the fact that the fall in D-glucose concentration coincides with an increased catabolism of endogenous amino acids, which are a source of unlabelled L-lactic acid in the islets [19,20].

[2- ^3H]Glycerol metabolism

At a low concentration of D-glucose (2.8 mM), the generation of ^3HOH from [2- ^3H]glycerol ($37\ \mu\text{M}$) was much higher ($P < 0.001$) in islets from GK than from control rats (Table 3). Even when expressed relative to the protein content of islets derived from the same preparation, the production of ^3HOH remained higher ($P < 0.001$) in GK rats ($1.14 \pm 0.12\ \text{pmol}/\mu\text{g}$ per 120 min) than in control animals ($0.49 \pm 0.03\ \text{pmol}/\mu\text{g}$ per 120 min). In both control and GK rats, the net production of tritiated acidic metabolites was about one order of magnitude lower than that of ^3HOH , in fair agreement with a previous observation [15].

When the concentration of D-glucose was raised from 2.8 to 16.7 mM, the conversion rate of [2- ^3H]glycerol into ^3HOH was increased significantly ($P < 0.005$ or less) in both control and GK rats. However, the relative magnitude of such an increase was much lower in GK than in control rats. Thus the 16.7 mM/2.8 mM D-glucose ratio of ^3HOH production did not exceed $128.9 \pm 8.1\%$ in GK rats, as compared ($P < 0.01$) with a control value of $165.7 \pm 9.9\%$ (d.f. = 38 in both cases).

Amino acid metabolism

Two approaches were used to investigate the catabolism of endogenous amino acids in the islets of control and GK rats.

The total production of NH_4^+ was first measured in islets incubated for 120 min in the absence or presence of 16.7 mM D-glucose. The basal production of NH_4^+ was higher ($P < 0.01$) in GK than in control rats (Table 4). Even when expressed relative to the paired protein content of islets derived from the same preparation, the basal values recorded in GK rats ($76.0 \pm 5.0\ \text{pmol}/\mu\text{g}$ per 120 min) remained significantly higher ($P < 0.03$) than those in control animals ($61.0 \pm 4.1\ \text{pmol}/\mu\text{g}$ per 120 min). D-Glucose (16.7 mM) significantly decreased NH_4^+ production in both control and GK rats ($P < 0.001$). The relative magnitude of such a sparing action was not significantly different ($P > 0.1$) in the two types of rats, the trend being towards a greater inhibition of NH_4^+ production by D-glucose in GK rats (glucose-induced decrement $47.7 \pm 9.8\%$; d.f. = 26) than in control animals (glucose-induced decrement $30.3 \pm 7.0\%$; d.f. = 26).

In the second approach, islets were preincubated for 30 min in the sole presence of L-[U- ^{14}C]glutamine (1.0 mM) and then incubated for another 30 min in the absence or presence of

Table 1 Metabolic status of control and GK rats

	Rats	
	Control	GK
Body wt. (g)	340.0 ± 6.1 (24)	304.3 ± 3.6 (24)
Plasma glucose (mM)	7.98 ± 0.13 (24)	17.06 ± 0.99 (24)
Plasma insulin ($\mu\text{-units/ml}$)	48.3 ± 5.7 (24)	70.9 ± 4.8 (24)
Islet protein content (ng/islet)	1900 ± 193 (8)	2410 ± 180 (8)
Islet insulin content ($\mu\text{-units/islet}$)	2020 ± 342 (8)	1109 ± 190 (8)

Figure 1 (a, b) Phase-contrast micrographs showing part of an islet from a control rat (a) or a GK rat (b); (c, d) electron micrographs showing part of a B-cell from a control rat (c) or a GK rat (d)

In (a), the clear spaces correspond to the extracellular space. In (b), vacuoles, arranged in circles or crescents, are apparent within the cytoplasm of several B-cells. The bar represents 10 μ m. In (c) and (d), as compared with the control, secretory granules appear more scarce, and the elements of the Golgi apparatus (G) are dilated in the GK rat. The bar represents 1 μ m.

Table 2 L-Lactic acid output by islets incubated for two successive periods of 10 min, first at 16.7 mM and then at 2.8 mM D-glucose

In some experiments the incubation medium also contained D-[U-¹⁴C]glucose.

		Lactate output (pmol/islet per 10 min)		
Rats		First incubation (0–10 min)	Second incubation (11–20 min)	Second/first incubation (ratio)
Control	Unlabelled lactate	95.6 $\pm$ 11.2 (10)	44.3 $\pm$ 4.9 (10)	0.50 $\pm$ 0.06 (10)
	L-[U- ¹⁴ C]Lactate	49.9 $\pm$ 11.9 (5)	15.0 $\pm$ 3.2 (5)	0.33 $\pm$ 0.05 (5)
GK	Unlabelled lactate	75.4 $\pm$ 12.5 (10)	33.9 $\pm$ 4.8 (10)	0.50 $\pm$ 0.06 (10)
	L-[U- ¹⁴ C]Lactate	50.5 $\pm$ 10.6 (5)	19.5 $\pm$ 2.7 (5)	0.44 $\pm$ 0.08 (5)

Table 3 Metabolism of [2-³H]glycerol (37 μ M)

	D-Glucose (mM)	Rats ...	Generation rate (fmol/islet per 120 min)	
			Control	GK
³ HOH	2.8		1172 $\pm$ 46 (20)	2847 $\pm$ 114 (20)
	16.7		1942 $\pm$ 107 (20)	3669 $\pm$ 199 (20)
Tritiated acidic metabolites	2.8		154 $\pm$ 16 (18)	227 $\pm$ 39 (20)
	16.7		213 $\pm$ 34 (20)	236 $\pm$ 31 (20)

Table 4 Production of NH₄⁺

D-Glucose (mM)	Rats ...	NH ₄ ⁺ production (pmol/islet per 120 min)	
		Control	GK
0		121.4 $\pm$ 9.1 (14)	165.6 $\pm$ 12.3 (14)
16.7		84.4 $\pm$ 6.5 (14)	92.0 $\pm$ 15.0 (14)

Table 5 Output of ¹⁴CO₂ from islets prelabelled with L-[U-¹⁴C]glutamine

The islet radioactivity (¹⁴C) content at the onset of incubation is expressed as L-glutamine equivalent by reference to the specific radioactivity of exogenous L-[U-¹⁴C]glutamine in the preincubation medium. The output of ¹⁴CO₂ over 30 min incubation is expressed relative to the paired islet radioactivity content. Metabolic poisons included KCN (5.0 mM), antimycin A (10 μ M) and rotenone (10 μ M). The ¹⁴C output/¹⁴C content ratios were corrected for the readings recorded in the presence of metabolic poisons and expressed relative to the mean basal value (no exogenous nutrient) found within the same experiment in the same type of rats.

	Rats	
	Control	GK
Islet radioactivity content (pmol/islet)	19.44 $\pm$ 0.77 (60)	28.36 $\pm$ 1.18 (60)
¹⁴ CO ₂ output/ ¹⁴ C content (%)		
No exogenous nutrient	14.27 $\pm$ 1.03 (12)	12.29 $\pm$ 1.04 (12)
BCH (10 mM)	23.56 $\pm$ 1.26 (12)	18.86 $\pm$ 0.89 (12)
3-Phenylpyruvate (10 mM)	21.14 $\pm$ 1.21 (12)	17.60 $\pm$ 0.78 (12)
D-Glucose (16.7 mM)	17.41 $\pm$ 0.90 (12)	14.50 $\pm$ 1.07 (12)
Metabolic poisons	3.09 $\pm$ 0.23 (12)	2.52 $\pm$ 0.30 (12)
Corrected ¹⁴ CO ₂ output/ ¹⁴ C content ratio (%)		
No exogenous nutrient	100.0 $\pm$ 3.6 (12)	100.0 $\pm$ 4.6 (12)
BCH (10 mM)	183.4 $\pm$ 6.1 (12)	173.1 $\pm$ 9.7 (12)
3-Phenylpyruvate (10 mM)	161.9 $\pm$ 6.6 (12)	159.3 $\pm$ 7.6 (12)
D-Glucose (16.7 mM)	128.6 $\pm$ 4.6 (12)	124.0 $\pm$ 5.8 (12)

unlabelled nutrient secretagogues or metabolic poisons (5.0 mM KCN, 10 μ M antimycin A and 10 μ M rotenone). At the onset of the final incubation, the radioactivity content of the islets, which corresponds mainly to L-[U-¹⁴C]glutamate [21], was higher ($P < 0.001$) in GK than in control rats (Table 5). Even when expressed relative to the paired protein content of islets taken from the same preparation, the radioactive content of the islets, computed as L-glutamine equivalent, remained higher ($P < 0.001$) in GK rats (10.20 $\pm$ 0.34 pmol/ μ g) than in control animals (7.74 $\pm$ 0.20 pmol/ μ g).

Table 6 Output of ¹⁴CO₂ from islets prelabelled with [U-¹⁴C]palmitate

The islet radioactivity (¹⁴C) content at the onset of incubation is expressed as palmitate equivalent by reference to the specific radioactivity of exogenous [U-¹⁴C]palmitate in the preincubation medium. The output of ¹⁴CO₂ over 120 min incubation is expressed relative to the paired islet radioactive content. Metabolic poisons included KCN (5.0 mM), antimycin A (10 μ M) and rotenone (10 μ M). The ¹⁴CO₂ output/¹⁴C content ratios were corrected for the readings recorded in the presence of metabolic poisons and expressed relative to the mean basal value (no exogenous nutrient) found within the same experiments in the same type of rats.

	Rats	
	Control	GK
Islet radioactivity content (pmol/islet)	1.56 $\pm$ 0.05 (48)	3.70 $\pm$ 0.28 (48)
¹⁴ CO ₂ output/ ¹⁴ C content (%)		
No exogenous nutrient	8.73 $\pm$ 0.49 (16)	8.39 $\pm$ 0.43 (16)
D-Glucose (16.7 mM)	7.29 $\pm$ 0.36 (16)	6.58 $\pm$ 0.40 (16)
Metabolic poisons	1.20 $\pm$ 0.12 (16)	1.12 $\pm$ 0.09 (16)
Corrected ¹⁴ CO ₂ output/ ¹⁴ C content ratio (%)		
No exogenous nutrient	100.0 $\pm$ 4.9 (16)	100.0 $\pm$ 5.0 (16)
D-Glucose (16.7 mM)	81.9 $\pm$ 4.9 (16)	75.2 $\pm$ 5.2 (16)

When the prelabelled islets were incubated in the absence of exogenous nutrient, the output of ¹⁴CO₂, expressed relative to their initial content, was not significantly different in control and GK rats. The absolute value for ¹⁴CO₂ output, expressed as L-glutamine equivalent, remained higher ($P < 0.06$), however, in GK rats (3.43 $\pm$ 0.24 pmol/islet per 30 min) than in control animals (2.83 $\pm$ 0.18 pmol/islet per 30 min).

As expected from previous reports [22,23], the output of ¹⁴CO₂ was markedly increased by either 2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH) or 3-phenylpyruvate and, to a lesser extent, by D-glucose, in either control or GK rats (Table 5). Relative to the basal value and after correction for the values recorded in the presence of metabolic poisons, the enhancing action of BCH, 3-phenylpyruvate and D-glucose on the ratio between ¹⁴CO₂ output and initial radioactivity content was not significantly different in control and GK rats (Table 5, lower panel). Nevertheless, the paired ratio between ¹⁴CO₂ output and initial radioactivity content was lower in nutrient-stimulated islets from GK than from control rats ($P < 0.05$ or less).

Palmitate metabolism

The contribution of endogenous fatty acid oxidation to respiration was judged in islets preincubated for 120 min with [U-¹⁴C]palmitate (0.33 mM) and D-glucose (8.3 mM) and then incubated for another 120 min in the presence or absence of unlabelled nutrient secretagogues or metabolic poisons (see above). At the onset of the final incubation, the radioactive content of the islets was higher ($P < 0.001$) in GK than in control rats (Table 6). Even when related to the paired protein content of islets obtained from the same preparation, the radioactive content remained higher ($P < 0.001$) in islets of GK rats (1.67 $\pm$ 0.11 pmol/ μ g) than of control animals (0.81 $\pm$ 0.03 pmol/ μ g).

Likewise, when the prelabelled islets were incubated for 120 min in the absence of exogenous nutrient, the output of ¹⁴CO₂, expressed as [U-¹⁴C]palmitate equivalent, was higher ($P < 0.001$) in GK rats (323 $\pm$ 46 fmol/islet per 120 min) than in control animals (141 $\pm$ 9 fmol/islet per 120 min). D-Glucose (16.7 mM), when present in the final incubation medium, significantly decreased ¹⁴CO₂ output from the prelabelled islets, in either

Table 7 Insulin release by islets from control and GK rats

Nutrient	Rats ...	Insulin release (μ -units/islet per 90 min)	
		Control	GK
Nil		55.2 $\pm$ 9.6 (20)	27.1 $\pm$ 2.6 (20)
BCH (10 mM)		100.1 $\pm$ 5.7 (20)	37.7 $\pm$ 4.4 (20)
3-Phenylpyruvate (10 mM)		115.4 $\pm$ 8.4 (20)	35.0 $\pm$ 5.1 (20)
D-Glucose (16.7 mM)		251.7 $\pm$ 12.4 (20)	86.1 $\pm$ 9.6 (20)

control or GK rats ($P < 0.02$ or less). Such a sparing action was not less marked in GK than control rats (Table 6, lower panel).

Insulin release

In the last series of experiments, we have examined the secretory response of pancreatic islets to the three major secretagogues used in the above metabolic studies, namely BCH, 3-phenylpyruvate and D-glucose (Table 7). Both the basal release of insulin and that evoked by each of these three nutrients were significantly lower in GK than control rats ($P < 0.01$ or less). The GK/control ratios for insulin output were not significantly different under the four experimental conditions listed in Table 7, and yielded an overall mean value of $37.8 \pm 3.2\%$ (d.f. = 152). In the islets of GK rats, the secretory response to BCH and 3-phenylpyruvate was thus as severely affected as that evoked by D-glucose. The increment in insulin output evoked by 3-phenylpyruvate in the islets of GK rats even failed to achieve statistical significance ($P < 0.2$).

When the mean secretory rate recorded in each experimental condition was expressed relative to the paired insulin content of islets derived from the same preparation, the ratio between insulin output and content was always lower in GK than in control rats. Pooling all available data, such a ratio only averaged in GK rats $63.7 \pm 6.0\%$ ($n = 8$; $P < 0.005$) of that recorded under the same experimental conditions in control rats ($100.0 \pm 6.4\%$; $n = 8$). This suggests that the impaired release of insulin cannot be blamed solely on the decreased hormonal content of the islets.

DISCUSSION

The present results indicate that the higher ATP/ADP ratio and lower K^+ conductance recently documented in islets of GK rats incubated at a low D-glucose concentration [8] cannot be attributed to stimulation of glycogenolysis. They coincide, however, with a higher catabolism of endogenous amino and fatty acids, as judged from both the production of NH_4^+ over 120 min incubation and the output of $^{14}CO_2$ from islets prelabelled with either L-[U- ^{14}C]glutamine or [U- ^{14}C]palmitate. Such an increased oxidation of the two major endogenous nutrients involved in the basal respiration of islets [19] may also account for the lower ratio between D-[6- ^{14}C]glucose and D-[2- ^{14}C]glucose oxidation previously documented in the islets of GK rats [6]. An accelerated catabolism of endogenous nutrients may indeed be expected to increase both the input of dicarboxylic acids into the tricarboxylic acid cycle and their later exit from such a cycle.

The high basal catabolism of endogenous amino acids may also explain, in part at least, the impaired secretory response of the islets of GK rats to either BCH or 3-phenylpyruvate. The insulinotropic action of these two secretagogues is indeed at-

tributable to an accelerated catabolism of endogenous amino acids, as a result of either the activation of glutamate dehydrogenase by BCH or the participation of 3-phenylpyruvate in transamination reactions [22,23]. Hence, a higher basal catabolism of endogenous amino acids may, within limits, impair the metabolic response to BCH or 3-phenylpyruvate. The ratio between $^{14}CO_2$ output and radioactive content in islets prelabelled with L-[U- ^{14}C]glutamine and then exposed to either BCH or 3-phenylpyruvate was indeed lower in GK than in control rats. An additional factor accounting for the poor secretory response of the islets to the last two secretagogues obviously consists in the lower insulin content of the islets. The ratio between insulin output and content was nevertheless lower in BCH- or 3-phenylpyruvate-stimulated islets from GK than from control rats.

The high oxidation rate of endogenous nutrients, the high ATP/ADP ratio [8], and the coinciding low ^{86}Rb fractional outflow rate [8] found at a low concentration of D-glucose (2.8 mM) in islets of GK as distinct from control rats, may conceivably account, in part at least, for the higher rate of 3HOH generation from [2- 3H]glycerol here also observed in islets prepared from GK rats and incubated at the same low hexose concentration. It is indeed known that the activation of the glycerol phosphate shuttle in islets stimulated by D-glucose or other nutrient secretagogues represents a Ca^{2+} -dependent process [15]. A limited activation of FAD-linked glycerophosphate dehydrogenase could thus occur in islets of GK rats as a result of the lower K^+ conductance and, hence, presumably higher Ca^{2+} inflow into B-cells of GK rats exposed to a low concentration of D-glucose. Along the same line of thinking, it should be underlined that, relative to the corresponding value found in control rats, the ratio between insulin output and content in GK rats was less severely affected ($P < 0.05$) in islets deprived of exogenous nutrient (GK/control rats: $83.7 \pm 5.2\%$) than in islets stimulated by BCH, 3-phenylpyruvate or D-glucose (GK/control rats: $57.0 \pm 5.6\%$).

A second anomaly in [2- 3H]glycerol metabolism consisted in the fact that a rise in hexose concentration from 2.8 to 16.7 mM caused a much more modest relative increment in 3HOH generation in GK than in control rats. This is consistent with the view that the altered activity of FAD-linked glycerophosphate dehydrogenase found in islet homogenates from GK rats participates in the impairment of glucose-stimulated insulin release [6,7].

In conclusion, therefore, the present findings, while revealing an abnormally high catabolism of endogenous nutrients in islets of GK rats, do not detract from the view that an inherited defect in B-cell FAD-linked glycerophosphate dehydrogenase contributes to the pathogenesis of B-cell dysfunction in this animal model of type-2 diabetes.

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