D-fructose metabolism and insulinotropic action in pancreatic islets: pathological aspects

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ABSTRACT
The main aim of the present review is to draw attention to the perturbations of D-fructose metabolism and insulinotropic action found in experimental models of dysfunction in insulin-producing cells. The first series of investigation was conducted in pancreatic islets of Goto-Kakizaki rats, an animal model of inherited type 2 diabetes. The second set of experiments deals with a comparable study performed on pancreatic islets from adult rats that had been injected with streptozotocin during the neonatal period, i.e. another current model of type 2 diabetes. Finally, the findings recorded in tumoral islet cells of the RINm5F line are presented.

KEYWORDS: D-fructose, insulin-producing cells, Goto-Kakizaki rats, streptozotocin-injected rats, tumoral islet cells of the RINm5F line

INTRODUCTION
In the two preceding articles in this series, available information on the physiological aspects of D-fructose metabolism and insulinotropic action in pancreatic islet cells was reviewed [1, 2]. The present and last article in the series deals with pathological aspects of the same biological processes in three distinct models of pancreatic islet cell dysfunction.

Goto-Kakizaki rats
The possible alterations in the metabolic and functional responses of pancreatic islets isolated from rats to D-fructose in animal models of islet dysfunction were first examined in Goto-Kakizaki (GK) rats [3-5]. These GK rats display a lower body weight, higher plasma D-glucose and insulin concentrations than those found in control rats of the same age and sex. However, the insulinogenic index, i.e. the paired ratio between plasma insulin and D-glucose concentration does not differ significantly in GK and control rats. Such is also the case with both the islet DNA content and DNA/protein ratio. The islet insulin content is lower, however, in GK rats than in control animals, whether expressed as ng/islet or relative to either the protein or DNA content of the islets [4, 5].

The basal value for insulin output recorded in the absence of any exogenous secretagogue was higher in islets from GK rats than in those from control rats [3, 5]. In a first study, the islets from GK rats released less insulin than those from control rats when incubated for 90 min in the presence of either D-glucose (11.1 or 80.0 mM), D-mannose (80.0 mM), D-fructose (240.0 mM), or both L-leucine and L-glutamine (10.0 mM each) [3]. The insulinotropic action of D-fructose appeared less severely affected than that of the other nutrient secretagogues. For instance, in the islets of GK rats exposed to 240.0 mM D-fructose, the output of insulin was virtually
11.1 mM D-glucose, at variance with the situation found under the same experimental conditions in the islets of control rats, in which case the insulin output from the islets exposed to the ketohexose was much lower (p < 0.001) than that evoked by the aldohexose [3]. Nevertheless, no significant difference between the secretory response to either D-glucose (11.1 mM) or D-fructose (240.0 mM) was observed anymore when the increments in insulin release above basal value evoked by either the hexose or ketose in GK rats were expressed relative to the corresponding values found in control animals, yielding an overall mean value of 11.9 ± 4.6% in GK rats as compared to 100.0 ± 6.5% in the control rats. A somewhat different picture was reached in a second series of experiments [5]. In the sole presence of 10.0 mM D-fructose, no significant increase in insulin output above basal value was observed, whether in control or GK rats. Hence, the secretory response to D-fructose was then judged from data collected at increasing concentrations of the ketose (10.0, 20.0, 30.0 mM) in the presence of D-glucose (10.0 mM). The release of insulin remained lower in GK rats than in control animals, when isolated pancreatic islets were incubated for 120 min in the presence of either 10.0 mM D-glucose, 10.0 mM 2-ketoisocaprate, or the combination of D-glucose (10.0 mM) and D-fructose (10.0, 20.0 or 30.0 mM). The impression was gained, under these experimental conditions, that the insulinotropic action of D-fructose was more severely affected than that of D-glucose in the islets of GK rats. First, in the islets from GK rats exposed to 10.0 mM D-glucose, D-fructose used at concentrations of 10.0 or 20.0 mM failed to significantly augment insulin output, at variance with the situation found in islets of control rats exposed to 10.0 or 20.0 mM D-fructose in the presence of 10.0 mM D-glucose. Second, in the presence of both D-glucose (10.0 mM) and D-fructose (30.0 mM), the output of insulin expressed relative to that recorded in the sole presence of D-glucose (10.0 mM) averaged 148 ± 14% in GK rats, compared to 194 ± 15% in control animals.

In two reports [4, 5], attention was also paid to metabolic variables in the islets of GK rats. As a rule, no significant difference was observed between control and GK rats. For instance, such was the case of the minor effects of D-fructose (10.0 mM), if any, on the generation of H2O from either D-[3-3H]glucose or D-[5-3H]glucose, the ratio between D-[5-3H]glucose and D-[3-3H]glucose utilization, the relative magnitude of the enhancing action of extracellular Ca2+ deprivation upon this ratio, the effects of D-glyceraldehyde (10.0 mM) upon the metabolism of either D-glucose or D-fructose (10.0 mM each) and the effect of D-mannoheptulose (20.0 mM) upon the metabolism of either D-glucose or D-fructose (10.0 mM each) in islets exposed concomitantly to the aldohexose and ketohexose. The ratio between D-[5-3H]fructose and D-[5-3H]glucose utilization, the ratio between D-[1-14C]glucose and D-[6-14C]glucose oxidation and the participation of the pentose phosphate pathway in the total metabolism of D-glucose whether in the absence or presence of D-fructose also failed to differ significantly in control and GK rats. Such was also the case for the relative magnitude of both the glucose-induced inhibition of D-[5-3H]fructose utilization and the glucose-induced increase in the paired ratio between D-[U-14C]fructose oxidation and D-[5-3H]fructose utilization, the concentration-related pattern for the enhancing action of D-glucose (5.0 to 40.0 mM) upon the paired ratio between D-[U-14C]fructose oxidation and D-[5-3H]fructose utilization, and the hierarchy for the relative magnitude of the enhancing action of D-glucose upon the oxidation of different 14C-labelled tracers of D-fructose (D-[1-14C]fructose, D-[3,4-14C]fructose and D-[6-14C]fructose) [4, 5]. Only a few significant differences were observed when comparing the data collected in GK rats and control animals. First, the paired ratio between the oxidation of D-[U-14C]glucose and utilization of either D-[3-3H]glucose or D-[5-3H]glucose was lower in GK rats than in control animals. In the first [5] and second [4] series of experiments, respectively, the ratio found in GK rats averaged to 89.5 ± 3.5 and 94.0 ± 2.0% of the mean corresponding values found in control animals (100.0 ± 3.2% and 100.0 ± 1.6%). Relative to the paired value for D-[5-3H]glucose utilization, the oxidation of either D-[1-14C]glucose or D-[6-14C]glucose (10.0 mM) by islets of GK rats also averaged to no more than 86.4 ± 1.8% of the mean corresponding control values (100.0 ± 1.4%) [5].
Likewise, the ratio between D-[U-14C]fructose oxidation and D-[5-3H]fructose utilization was, as a rule, lower in GK rats than in control animals, whether in the absence of D-glucose or presence of the aldohexose (5.0 to 40.0 mM) [5]. As judged from the GK/control ratio for the oxidation of D-[U-14C]fructose, on one hand, and the utilization of either D-[3-3H]fructose or D-[5-3H]fructose, on the other hand, a preferential impairment of oxidative events in the islets of GK rats was also observed in the second study [4]. Such a view was further supported by the finding that the generation of acidic metabolites from D-[U-14C] fructose, which was close to the limit of detection in control rats, always yielded sizeable readings in GK rats [4]. A last anomaly found in the islets of GK rats relates to the fraction of D-fructose metabolized to CO2 and D-glyceraldehyde 3-phosphate via the pentose phosphate pathway. In the islets of control rats, such a fraction, which was at least twice higher than that recorded for the fate of D-glucose in islets exposed to an equimolar concentration of D-glucose, was indeed decreased in the concomitant presence of the ketohexose and aldohexose (10.0 mM each) [6]. At variance with the situation found in control rats, however, D-glucose (10.0 mM) failed to decrease the fractional contribution of the pentose phosphate pathway to the catabolism of D-fructose in the islets of GK rats [5]. Incidentally, in a parallel study dealing with the D-[3-3H] glucose/D-[5-3H]glucose utilization ratio in islets from control and GK rats incubated for 120 min in the presence of either 2.8 or 16.7 mM D-glucose, the rise in D-glucose concentration significantly increased this ratio in the islets from control rats but failed to do so in the islets from GK rats [7]. In the islets of control rats, the partial escape from detritiation of [1-3H]glycerone-3-phosphate generated from D-[3-3H]glucose coincided with a much higher tritiation of islet lipids in the islets exposed to D-[3-3H]glucose as distinct from D-[5-3H] glucose. No significant difference between control and GK rats was observed when the labeling of islet lipids, which is mainly attributable to the labeling of their glycerol moiety rather than fatty acid moiety, was measured after 120 min incubation in the presence of either 2.8 or 16.7 mM D-[1-14C]glucose, D-[6-14C]glucose or D-[3-3H] glucose [8].

Adult rats injected with streptozotocin during the neonatal period

A comparable investigation was then conducted in islets obtained from either control rats or animals that had been injected with streptozotocin (STZ) during the neonatal period [9]. The control and STZ rats used in this study were of comparable age and body weight. The STZ rats displayed a higher plasma D-glucose concentration, lower plasma insulin concentration, lower insulogenic index and lower islet insulin content than the control animals.

The basal insulin output and that recorded in the presence of D-glucose (10.0 mM), D-fructose (10.0 mM), both D-glucose and D-fructose (10.0 mM each) or 2-ketoisocaprate (10.0 mM) were always significantly lower in STZ rats than in control animals. In both STZ rats and control animals, D-glucose (10.0 mM), the association of D-glucose and D-fructose (10.0 mM each), and 2-ketoisocaprate (10.0 mM), but not D-fructose alone (10.0 mM), augmented insulin release above basal value. Whilst D-fructose (10.0 mM) significantly augmented insulin release evoked by D-glucose (also 10.0 mM) in the islets from control rats, such was not the case in the islets from STZ rats.

At a 10.0 mM concentration, the oxidation of D-[U-14C]glucose, the utilization of D-[5-3H] glucose and the paired ratio between these two variables were much lower in the islets of STZ rats than in those of control animals, whether in the absence or presence of D-fructose (10.0 mM), which did not affect significantly the catabolism of D-glucose whether in control or STZ rats. The oxidation of D-[U-14C]fructose, utilization of D-[5-3H] fructose and paired ratio between these two variables were also lower in STZ rats than in control animals. As judged from the mean values for each variable, the relative magnitude of the decrease in D-fructose metabolism recorded in STZ rats was less severe than that found for D-glucose metabolism, the former decrease in D-fructose metabolism averaging to 64.2 ± 4.0% (p < 0.02) of the corresponding value (100%) for the latter decrease in D-glucose metabolism.

A salient finding in this study merits to be underlined. At variance with the situation prevailing in the islets from control rats, D-glucose (10.0 mM)
failed to increase the oxidation of D-[U-14C]fructose, D-[1-14C]fructose or D-[6-14C]fructose in the islets from STZ rats [9].

**Tumoral insulin-producing cells**

Based on earlier studies, the metabolic and functional responses to D-fructose in tumoral insulin-producing cells of the RINm5F line differed in some respects from those otherwise observed in normal islet cells.

The phosphorylation of D-[U-14C]fructose by RINm5F cell homogenates indicated that the phosphorylation of the ketohexose was catalyzed, in these tumoral cells like in normal islet cells, by hexokinase rather than fructokinase [10]. Thus, the phosphorylation of the ketohexose by RINm5F cell homogenates, was virtually identical in the absence or presence of KCl (200.0 mM). At increasing concentrations of the ketohexose (1.0-30.0 mM), its phosphorylation rate by RINm5F cell homogenates yielded, in a double reciprocal plot, a straight line with a maximal velocity close to 4.2 pmol/min for 10^5 cells and a K_m close to 4.0 mM. D-glucose (2.8 mM) inhibited the phosphorylation of D-[U-14C]fructose (16.7 mM) by 82.9 ± 1.5%, this being compatible with a 40-times greater affinity of hexokinase for D-glucose than D-fructose. The product of D-fructose phosphorylation by RINm5F cell homogenates was identified as D-fructose 6-phosphate through exposure to both phosphoglucose isomerase and glucose-6-phosphate dehydrogenase in the presence of NADP⁺ [10].

A first unexpected finding concerned the uptake of the ketohexose by intact RINm5F cells [11]. Whether at low (2.8 mM) or high (16.7 mM) concentration, the apparent distribution space of D-[U-14C]fructose, in excess of that of L[1-14C]glucose (2.0 mM) used as an extracellular marker, was much lower than that of D-[U-14C]glucose, D-[U-14C]mannose or D-[U-14C]galactose, representing no more than about one third of the close-to-equal values recorded with the other three hexoses [11].

A model was proposed for the catabolism of D-fructose in RINm5F cells. It was based on such measurements as the incorporation of D-[U-14C]fructose in glycogen, the utilization of D-[5-3H]fructose, the oxidation of D-[1-14C]fructose, D-[6-14C]fructose or D-[U-14C]fructose, the output of unlabelled pyruvate and lactate, the generation of 14C-labelled lactate, as well as that of 14C-labelled pyruvate and its other metabolites (mainly 14C-labelled amino acids) in RINm5F cells incubated for 120 min in the presence of D-[U-14C]-fructose (16.7 mM). A salient finding consisted in the fact that D-fructose 6-phosphate generated from exogenous D-fructose was largely channeled into the pentose cycle, only about 23% of the pentose phosphates formed in this pathway being diverted from the pentose cycle. When the same experiments were conducted in the concomitant presence of D-glucose (2.8 mM), the phosphorylation of the ketohexose was decreased by about 40%, the amount of D-fructose 6-phosphate generated from exogenous D-fructose and oriented into the pentose phosphate pathway being decreased by about 90% [10].

When tested at a concentration of 16.7 mM, all hexoses (D-glucose, D-mannose, D-galactose) except D-fructose augmented significantly the uptake of O2 by the RINm5F cells [12]. The effects of these hexoses on the oxidation of endogenous nutrients were also investigated [11]. Their sparing action upon 14CO2 output from cells preincubated for 30 min in the presence of L-[U-14C]glutamine (1.0 mM), as expressed in percent of the mean control value found during a 30 min final incubation conducted in the absence of exogenous nutrient, averaged 34 ± 3% in the case of D-glucose, 32 ± 2% in the case of D-mannose, 14 ± 3% in the case of D-fructose and 4 ± 4% in the presence of D-galactose, all hexoses being used at a 16.7 mM concentration. Likewise, after 120 min preincubation in the presence of [U-14C]palmitate (0.3 mM) and unlabelled D-glucose (8.3 mM), the sparing action on 14CO2 output from the prelabelled cells over a final incubation of 120 min, again expressed in percent of the mean reference value found in the absence of exogenous nutrient, averaged 34 ± 3% in the case of D-glucose, 32 ± 2% in the case of D-mannose, 14 ± 3% in the case of D-fructose and 4 ± 4% in the presence of D-galactose, all hexoses being used at a 16.7 mM concentration. Likewise, after 120 min preincubation in the presence of [U-14C]palmitate (0.3 mM) and unlabelled D-glucose (8.3 mM), the sparing action on 14CO2 output from the prelabelled cells over a final incubation of 120 min, again expressed in percent of the mean reference value found in the absence of exogenous nutrient, averaged 56 ± 5 and 60 ± 4% in the case of D-glucose and D-mannose, respectively, as distinct from 24 ± 3 and 25 ± 5% in the case of D-fructose and D-galactose, respectively [11].

The effects of the same four hexoses (16.7 mM) on cell lipid labeling were then explored [12]. Over 120 min incubation, D-fructose and D-galactose failed to augment the incorporation of 3HOH into either the fatty acid or glycerol
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After 30 min incubation, the mean ATP content was higher and the mean ADP content lower in RINm5F cells exposed to D-glucose, D-mannose, D-fructose or D-galactose (16.7 mM each) than in cells deprived of exogenous nutrient. The ATP/ADP ratio was increased from a basal value of 2.43 ± 0.14 to mean values ranging from 2.94 ± 0.15 to 4.39 ± 0.46 [11]. Based on the measurements of relevant variables, the estimated hexose-induced increase in ATP generation rate was again lower in cells exposed to either D-fructose or D-galactose than in the cells exposed to either D-glucose or D-galactose [11, 12].

In agreement with the metabolic data, D-fructose and D-galactose were less potent than either D-glucose or D-mannose, all tested at a 16.7 mM concentration, in stimulating insulin release above basal value, whether in the absence or presence of theophylline (1.4 mM) [11]. Likewise, whether at a concentration of 2.8 or 16.7 mM, the stimulation of protein biosynthesis in the RINm5F cells, as judged from the incorporation of L-[4-3H]phenylalanine into TCA-precipitable material by D-fructose or D-galactose, was much lower than that found with either D-glucose or D-mannose [12]. D-fructose (16.7 mM) exerted very little, if any, effect upon the biosynthetic response of the RINm5F cells to increasing concentrations of D-glucose, as little as 50 μM D-glucose being sufficient to increase protein biosynthesis in RINm5F cells preincubated for 60 min in the absence of exogenous nutrient. In the light of these findings, the effect of D-fructose (16.7 mM) upon the production of 3H2O from D-[5-3H]glucose and that of 14CO2 from D-[U-14C]glucose was examined in RINm5F cells exposed to low concentrations of the aldohexose (25 and 50 μM). Both metabolic variables were significantly higher in the presence of 50 μM as distinct from 25 μM D-glucose, whilst D-fructose failed to significantly affect D-glucose utilization or oxidation under these experimental conditions [12].

CONCLUSION

The present review emphasizes the versatility of perturbations in the metabolic and functional responses of insulin-producing cells to D-fructose, encountered in distinct models of pancreatic islet cell dysfunction.

CONFLICT OF INTEREST STATEMENT

No conflict of interest.

REFERENCES