Lactate Production in Pancreatic Islets

Jorge Tamarit-Rodriguez, Lars-Åke Idahl, Elena Giné, Oscar Alcazar, and Janove Sehlin

Lactate production, glucose utilization, glucose oxidation, and insulin release were studied in islets from rat and *ob/ob* mice. Lactate was determined with a highly sensitive method, based on esterification, subsequent separation, and quantitation with high-performance liquid chromatography. There was a significant lactate production in the absence of glucose, which increased with glucose concentrations up to 3 mmol/l, reaching its half-maximal rate in the presence of 0.2–1.0 mmol/l glucose in both species. Glucose utilization displayed a wider glucose concentration dependence, with a $K_{0.5}$ value between 3 and 10 mmol/l glucose. The rates of glucose utilization and lactate production were similar at 3 mmol/l glucose in rat islets and at about 6 mmol/l glucose in *ob/ob* mice islets. Saturation of lactate production at low glucose concentrations is probably contributing to the observed preferential stimulation of oxidative metabolism at higher concentrations. D-Mannoheptulose caused a marked inhibition of glucose utilization and glucose oxidation at 20 mmol/l glucose in islets from rat or *ob/ob* mice, as would be expected from a competitive inhibition of glucokinase. By contrast, D-mannoheptulose reduced only marginally the islet metabolism at 3 mmol/l glucose, which is consistent with an effective mannoheptulose-induced inhibition of the glucokinase-dependent, minor part of glucose phosphorylation at this low glucose concentration. *Diabetes* 47:1219–1223, 1998

It is now generally accepted that the β-cell signaling system is metabolic in nature. Glucose, the main physiological stimulus for insulin secretion, has to be metabolized; and its metabolism generates signals (increased cytosolic ATP or others) that couple it to exocytosis (1–3). Accumulated experimental evidence first suggested that the metabolism of glucose through glycolysis might provide the necessary messengers (protons, adenine nucleotides, or reduced pyridine nucleotides) responsible for secretory activation (4). Later on, interest has been focused on the interplay between glycolysis and mitochondrial metabolism in order for glucose to stimulate secretion (5). Furthermore, a preferential stimulation of aerobic glycolysis relative to total glycolysis has been shown to occur when islet cells are challenged by glucose (6,7).

It has been suggested that the preferential stimulation of the oxidative metabolism of glucose could partially be attributed to a calcium-induced activation of mitochondrial glycerol 3-phosphate dehydrogenase and the resultant acceleration of cytosolic NADH reoxidation by mitochondria (7). Lactate formation may compete with the other known shuttle systems for the reoxidation of cytoplasmic NADH. Lactate's rate of production determines what is known as the anaerobic glycolytic flux. Previous attempts to measure lactate production have utilized indirect approaches. Thus, anoxia has been shown to decrease islet glucose utilization (6,8), which was attributed to a relatively low activity of lactate dehydrogenase (6). This finding is in contrast with reported values of lactate dehydrogenase activity in islet homogenates, which by far exceeded the maximum glycolytic flux in intact islets (9,10). On the other hand, it was recently shown that lactate dehydrogenase activity is much lower in β-cells than in non–β-cells, insulin-secreting tumoral cells insensitive to glucose, or liver cells, whereas the converse was true for mitochondrial glycerol 3-phosphate dehydrogenase (11).

The balance between aerobic and anaerobic glycolysis reflects the proportion of mitochondrial oxidation and the cytoplasmic lactate dehydrogenase reduction of pyruvate, respectively. We studied this balance by measuring lactate production, glucose oxidation, and overall glucose utilization at different glucose concentrations. Previous reports showed considerable variation between both the absolute amount of lactate produced and the concentration dependence on glucose (12,13). In this study, we used islets from rats—to permit comparisons to previous experiments in this area—and from *ob/ob* mice, because they are extremely rich in β-cells (14). A direct method was used for the quantitation of lactate, based on high-performance chromatographic separation after derivatization of lactate into a compound with high molar extinction coefficient (15).

RESEARCH DESIGN AND METHODS

Materials. D-[5-^{3}H]glucose, D-[U-^{14}C]glucose, [^{3}H]H_2O, NaH-[^{14}C]CO_3, and Na^{125}I were from DuPont de Nemours (Germany) or Amersham Iberica S.A. (Spain). D-Mannoheptulose, 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane), and 4-bromophenacylbromide were from Fluka Chemie A.G. (Switzerland). D-Lactate (monolithium salt), sodium pyruvate, bovine serum albumin, and collagenase P were from Boehringer Mannheim (Germany). All other reagents (analytical grade) and organic solvents (acetonitrile, gradient grade) were from E. Merck (Germany).

Methods. Islets were isolated by collagenase digestion (16) of the pancreas of Wistar rats (males of 250 g body wt fed ad libitum) or by free-hand microdissection of pancreas from *ob/ob* mice fasted overnight (17). Rat islets were incubated in Krebs-Ringer solution buffered with 20 mmol/l HEPES and 5 mmol/l $NaHCO_3$, containing 0.5% bovine serum albumin. *Ob/ob* mice islets were incubated in the same type of medium without sodium bicarbonate and albumin. In control experiments, the addition of sodium bicarbonate or albumin at these concentrations did not affect islet glucose metabolism. The ratio of islets to incubation medium was constant in all the metabolic studies (1 rat islet/1 µl or 1 *ob/ob* islet/10 µl). In experiments aimed at studying insulin secretion, this ratio was changed to 1 rat islet/100 µl. Insulin in the incubation medium was measured radioimmunologically (18).

The amount of lactate accumulated in the incubation medium after 120 min at 37°C was measured with an established high-performance liquid chromatography (HPLC) method (15) that was slightly modified as follows. Aliquots

From the Department of Biochemistry (J.T.-R., E.G., O.A.), School of Medicine, Complutense University, Madrid, Spain; and the Department of Histology and Cell Biology (L.-Å.I., J.S.) Umeå University, Umeå, Sweden.

Address correspondence and reprint requests to Jorge Tamarit-Rodriguez, Universidad Complutense, Facultad de Medicina, Departamento de Bioquímica, Madrid-28040 Spain. E-mail: tamarit@eucmax.sim.ucm.es.

Received for publication 8 January 1998 and accepted in revised form 23 April 1998.

HPLC, high-performance liquid chromatography.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/47/8/1219/364628/47-8-1219.pdf by guest on 29 February 2024

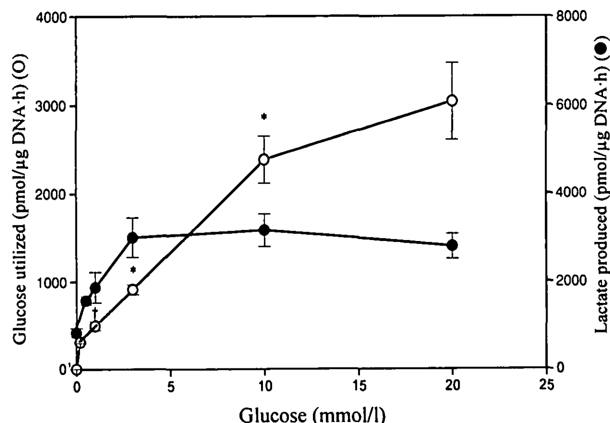


FIG. 1. Dose dependence of glucose utilization (○) and lactate (●) production on glucose in islets from *ob/ob* mice. Batches of five islets were incubated at 37°C for 120 min. Glucose utilization was measured as the production of [$^3H_2$]O from D-[5-^{3}H]glucose. Lactate accumulated in the incubation medium was determined by an HPLC method as described in METHODS. The data points show mean values ± SE for 6–18 observations. *P < 0.001, †P < 0.01 compared with the glucose concentration immediately below.

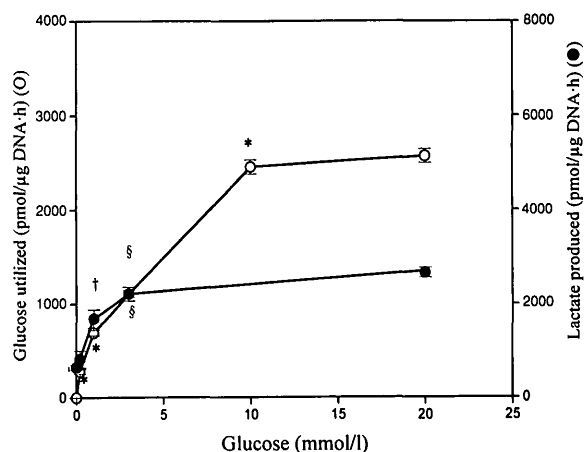


FIG. 2. Dose dependence of glucose utilization (○) and lactate (●) production on glucose in rat islets. Batches of 20 (utilization) or 30 (lactate) islets were incubated at 37°C for 120 min. Other experimental details were as described in Fig. 1. The data points show mean values ± SE for 3–33 observations. *P < 0.001, †P < 0.01, §P < 0.05 compared with the glucose concentration immediately below.

(25 μl) of the incubation medium from rat islet incubations were deproteinized with 1.35 mol/l $HClO_4$ (20 μl), and the excess $HClO_4$ was precipitated and neutralized with 0.1 mol/l Tris base and 2.8 mol/l $KHCO_3$ (15 μl). After centrifugation, an aliquot of the supernatant (40 μl) was desiccated under vacuum overnight after addition of 200 mmol/l NaOH (20 μl) to favor the formation of sodium lactate. Aliquots (20 μl) of incubation medium from *ob/ob* islet incubations, which did not contain albumin, were directly desiccated after addition of 50 mmol/l NaOH (10 μl). The dried residue was extracted with 200 μl acetonitrile containing 5 (rat) or 15 (*ob/ob* mouse) nmol/l of 18-crown-6 as a catalytic agent for the solubilization of lactate in the organic phase and 100 (rat) or 300 (*ob/ob* mouse) nmol/l of 4-bromophenacyl bromide for its esterification with lactate. After 10 min of gentle shaking, the derivatization reaction was allowed to proceed for 20 min at 80°C in tight, screw-capped vials. The lactate ester formed was then isocratically separated (retention time of ~9 min) on a reversed-phase C18-column (4 μm, 8 × 100 mm) with acetonitrile:water (30/70, vol/vol) and quantitated by its absorbance at 260 nm. The HPLC system was from Waters (Massachusetts) and included a W600 MSDS-module for solvent delivery, a U6K manual injector, a 484 absorbance detector, and a M746 model recorder. D-Lactate (monolithium salt) dissolved in the incubation medium (25–500 μmol/l) and treated as a sample was used as standard in each determination. In accordance with the original report of this method (15), we found that pyruvate was not significantly derivatized; it did not interfere with lactate derivatization at concentrations as high as 10 mmol/l in the incubation medium.

To detect possible pyruvate release by rat islets (60 islets/60 μl) into the incubation medium, two aliquots (20 μl each) of incubation medium (after 120 min incubation at 20 mmol/l glucose) were mixed with 5 μl reagent containing 5 mmol/l NADH and 54 U/l lactate dehydrogenase. One sample was incubated for 120 min at 37°C, and the other was directly deproteinized without incubation. The lactate content in the two samples was measured with the HPLC method. Pyruvate and D-lactate (25–400 μmol/l) in incubation medium were used as standards and were treated as the samples (vide supra). These control experiments showed a stoichiometric conversion of pyruvate to lactate.

The rates of glucose utilization and oxidation were measured as the production of [$^3H_2$]O and [^{14}C]O_2 from D-[5-^{3}H]glucose and D[U-^{14}C]glucose, respectively (19). The recovery of externally added [$^3H_2$]O and NaH[^{14}C]O_3 was routinely checked and was used to correct the metabolic rates accordingly.

DNA was measured in the rat islets after incubation. Most of the remaining medium was aspirated, and the islets were washed twice with 5 mmol/l NaOH (100 μl) and sonicated in a final volume of 100 μl. Incubated islets from *ob/ob* mice were separated from the medium, frozen in isopentane chilled with liquid nitrogen, freeze-dried, and finally weighed on a quartz-fiber micro-balance. The preweighed islets were then sonicated in 100 μl of 5 mmol/l NaOH. Aliquots of sonicated rat (40 μl) or *ob/ob* mice (20 μl) islets were then used for the fluorometric determination of DNA (20).

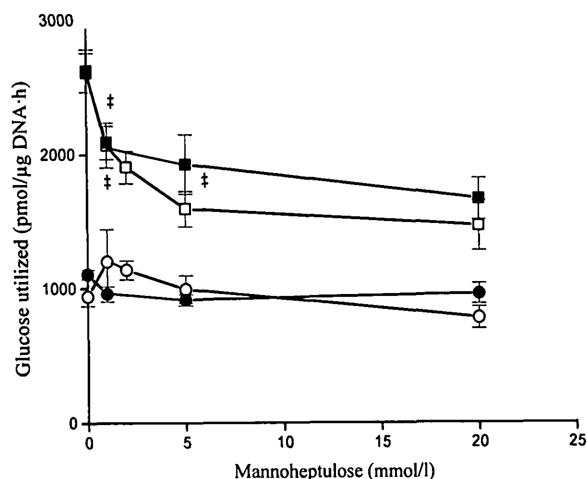
Statistical comparisons were performed with non-paired, two-tailed Student's *t* test. All the experimental data are presented as the mean values ± SE, and the numbers of separate experiments are given in parentheses.

RESULTS

Figures 1 and 2 show the concentration-response relationships for glucose-dependent lactate production and for glucose utilization in rat and *ob/ob* mice islets. In both animal species, there was an hyperbolic increase of lactate production from glucose in the range of 0–3 mmol/l. Elevating the glucose concentration above 3 mmol/l produced no further increment (*ob/ob* mice islets) or a small increase (rat islets) in the rate of lactate production, which reached its half-maximum value between 0.2 and 1.0 mmol/l glucose in both types of islets. Glucose utilization increased over a wide concentration range (0–20 mmol/l), with a $K_{0.5}$ value between 3 and 10 mmol/l glucose in both types of islets. The rate of lactate production markedly exceeded the glucose utilization rate when rat islets were incubated in <3 mmol/l glucose. For the *ob/ob* mouse, the corresponding crossing-over point seemed to be around 5–6 mmol/l glucose in the medium. The discrepancy between the rates of lactate production and glucose utilization at low glucose concentrations was most pronounced for *ob/ob* mice islets. Pyruvate could not be detected in the medium even after the rat islets had been incubated for 120 min at 20 mmol/l glucose (–22 ± 107 pmol pyruvate/h and μg DNA; n = 9).

D-Mannoheptulose is a known competitive inhibitor of glucose phosphorylation in islet homogenates (21–23) and was therefore used in attempts to dissect the relationship between fluxes in glucose metabolism and lactate production. Figure 3 shows that D-mannoheptulose decreased the utilization of 20 mmol/l glucose in both rat and *ob/ob* mice islets in a concentration-dependent manner. A close to maximum effect was obtained at 5 mmol/l D-mannoheptulose. Half-maximal effect was obtained at a D-mannoheptulose concentration around 1 mmol/l. High D-mannoheptulose concentrations (20 mmol/l) reduced the utilization rates of 20 mmol/l glucose to levels 50–70% higher than those obtained at 3 mmol/l glucose in the absence of the inhibitor (Table 1). D-Mannoheptulose did not significantly modify the utilization of 3 mmol/l glucose in either rat or *ob/ob* mice islets.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/47/8/1219/364628/47-8-1219.pdf by guest on 29 February 2024



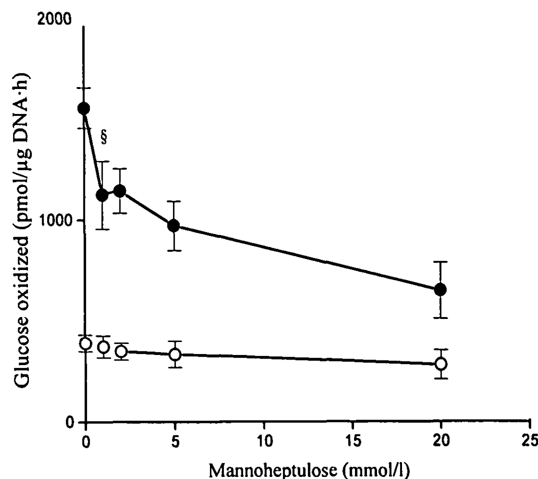


FIG. 3. Dose dependence of glucose utilization on mannoheptulose in *ob/ob* mice (□,○) or rat (■,●) islets in the presence of 3 (●,○) or 20 (■,□) mmol/l glucose. Two or three batches, each consisting of 5 (*ob/ob* mice) or 20 (rat) islets, were incubated at 37°C for 120 min. Glucose utilization was determined as the production of [$^3H_2$]O from D-[5-^{3}H]glucose. The data points show mean values ± SE for 4–33 observations. ‡P < 0.02 compared with the D-mannoheptulose concentration immediately below.

FIG. 4. Dose dependence of glucose oxidation on mannoheptulose in *ob/ob* mice islets in the presence of 3 (○) or 20 (●) mmol/l glucose. Two or three batches, each consisting of five islets, were incubated at 37°C for 120 min. Glucose oxidation was determined as the production of [^{14}C]O_2 from D-[U-^{14}C]glucose. The data points show mean values ± SE for six to eight observations. §P < 0.05 compared with the D-mannoheptulose concentration immediately below.

The rate of glucose oxidation at 3 mmol/l glucose was three times higher in *ob/ob* mice islets than in rat islets and was stimulated fourfold in both islet species by increasing the sugar concentration from 3 to 20 mmol/l (Table 1). The oxidation of 20 mmol/l glucose was highly sensitive to D-mannoheptulose with half-maximal inhibition at 1–3 mmol/l mannoheptulose in *ob/ob* mice islets (Fig. 4). However, the oxidation of 3 mmol/l glucose was not significantly modified by D-mannoheptulose in *ob/ob* mice islets at any of the tested concentrations of the inhibitor. In rat islets, 20 mmol/l D-mannoheptulose induced a large, significant decrease of glucose oxidation at 20 mmol/l. It also reduced glucose oxidation at 3 mmol/l hexose, with a low level of statistical significance (P < 0.05).

Figure 5 shows that D-mannoheptulose decreased lactate production from 20 mmol/l glucose in a dose-dependent manner in *ob/ob* mice islets. At 20 mmol/l, the aldoheptose caused a 37% reduction. However, the decrease of islet lactate production reached statistical significance only at concentrations of D-mannoheptulose of 5 mmol/l or higher. For comparison, the equivalent data for D-mannoheptulose effects on glucose oxidation and utilization, presented in Figs. 3 and 4, have also been inserted in Fig. 5. D-Mannoheptulose (20 mmol/l) decreased the lactate production from rat islets in the presence of 3 as well as 20 mmol/l glucose (Table 1).

Glucose-stimulated insulin secretion, like glucose metabolism, was very sensitive to D-mannoheptulose, which induced a half-maximal inhibition around 2 mmol/l (Fig. 6). D-Mannoheptulose at 20 mmol/l completely inhibited the secretory response to glucose (not statistically different from the secretory rate observed at 3 mmol/l glucose).

TABLE 1
Effect of D-mannoheptulose (MH) on glucose utilization, glucose oxidation and lactate production by rat or *ob/ob* mice islets

Addition to medium		Metabolic parameter		
Glucose (mmol/l)	MH	Glucose utilization	Glucose oxidation (pmol · µg DNA^{-1} · h^{-1})	Lactate production
ob/ob mice islets				
3	—	942 ± 70 (11)	392 ± 41 (8)	ND
3	20	782 ± 82 (11)	283 ± 71 (8)	ND
20	—	2,626 ± 159 (13)	1,559 ± 100 (6)	3,629 ± 357 (9)
20	20	1,466 ± 184 (13)*	649 ± 139 (6)*	2,275 ± 167 (9)†
Rat islets				
3	—	1,107 ± 36 (33)	124 ± 9 (17)	3,780 ± 197 (12)
3	20	961 ± 76 (5)	78 ± 15 (4)‡	2,633 ± 179 (14)‡
20	—	2,612 ± 146 (6)	507 ± 33 (20)	3,576 ± 153 (15)
20	20	1,666 ± 153 (7)†	246 ± 20 (4)*	2,741 ± 177 (15)†

Data are means ± SE obtained from the number of separate experiments given in parentheses. One to three batches, each consisting of 5 (*ob/ob* mice) or 20 (rat) islets, or in the case of lactate, 5 (*ob/ob* mice) or 30 (rat) islets, were incubated at 37°C for 120 min. Assay procedures for the metabolic parameters were described in METHODS. P values for difference from control without D-mannoheptulose are as follows: *P < 0.001, †P < 0.01, ‡P < 0.05. ND, not determined.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/47/8/1219/364628/47-8-1219.pdf by guest on 29 February 2024

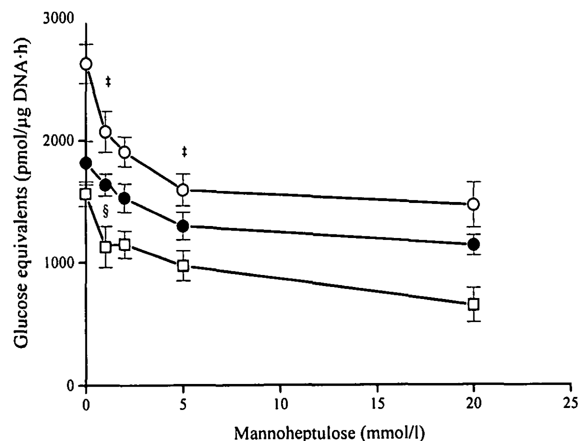


FIG. 5. Dose dependence of glucose utilization (○), glucose oxidation (□), and lactate production (●) on mannoheptulose in *ob/ob* mice islets in the presence of 20 mmol/l glucose. One to three batches, each consisting of 5 (*ob/ob* mice) or 20 (rat) islets or, in the case of lactate, 5 (*ob/ob* mice) or 30 (rat) islets, were incubated at 37°C for 120 min. Glucose utilization and glucose oxidation were determined as described in the legends to Figs. 3 and 4. Lactate was measured in an aliquot of the incubation medium as described in METHODS. The data points show mean values ± SE for 6–13 observations. ‡$P < 0.02$, §$P < 0.05$ compared with the D-mannoheptulose concentration immediately below.

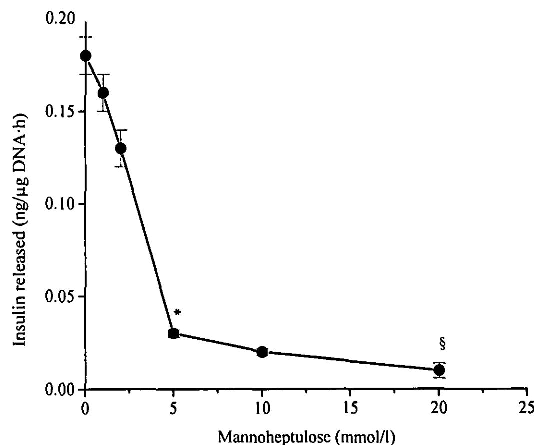


FIG. 6. Dose dependence of insulin release on mannoheptulose in rat islets in the presence of 20 mmol/l glucose. Two or three batches, each consisting of 10 islets, were incubated at 37°C for 120 min. Insulin was measured with a radioimmunoassay in an aliquot of the incubation medium as described in METHODS. The data points show mean values ± SE for 8–26 observations. *$P < 0.001$, §$P < 0.05$ compared with the D-mannoheptulose concentration immediately below.

DISCUSSION

Lactate production already reached a maximum at low glucose concentrations in islets of both rat and *ob/ob* mice. Maximum rates were obtained around 3 mmol/l. The $K_{0.5}$ values were about 0.5 mmol/l glucose. This is in clear contrast to some previous studies that reported much higher $K_{0.5}$ values: 7 mmol/l (22), 8.3 mmol/l (23), and 15 mmol/l (24). The differences may, at least to some extent, be ascribed to the different methods employed for lactate determination. The present method is based on HPLC separation of a lactate derivative and has a sensitivity limit in the picomolar range (15). Furthermore, it does not rely on the relative nonspecificity of lactate dehydrogenase that seems to overestimate the conversion of glucose into lactate in islets (25). However, it should be noted that when the enzymatic lactate assay was used, there was also no significant difference between the rate of lactate production at 3 and 20 mmol/l glucose (25). The lowest $K_{0.5}$ value (2 mmol/l) previously reported for glucose-induced lactate production by rat islets was obtained when lactate was measured with a LDH method amplified by enzymatic cycling of the formed NADH (26). The maximum rates of islet lactate production in the presence of glucose vary markedly among previous reports, ranging from 30 to 120 pmol lactate per islet and hour (22–29, reviewed in 12). As several of these studies have expressed their values as per islet, the varying lactate levels may be attributable, at least in part, to different islet size. Involvement of other endocrine cells to some extent cannot be disregarded. The fact that the extremely β-cell–rich *ob/ob* mice islets showed levels of lactate production similar to those of the rat islets strongly suggests that the data are representative for the β-cells in the islets.

It has been proposed that the rate of lactate production from exogenous glucose in intact islet cells is not restricted by the activity of lactate dehydrogenase (10). Our present results do not indicate whether the saturation of lactate production at low glucose depends on a particularly low lactate dehydrogenase activity or merely restriction of the supply of substrates.

Glucose utilization by islets of both rat and *ob/ob*-mice showed higher $K_{0.5}$ values, between 3 and 10 mmol/l glucose, compared with the values for lactate production. The maximum rate of lactate formation approximated the rate of utilization at 3 (rat islets) or 6 (*ob/ob* islets) mmol/l glucose. One may thus conclude that at substimulatory glucose concentrations, only a minor fraction of the glucose residues proceed to oxidative degradation. Increasing the glucose concentration to 20 mmol/l produces a preferential increase of glucose oxidation because lactate production remains constant. *Ob/ob* mice islets showed a substantially higher rate of oxidative metabolism at low glucose concentrations. However, at higher glucose concentrations, *ob/ob* mice islets also showed a preferential stimulation of glucose oxidation over lactate production. The observation that the lactate production was larger than the glucose utilization at low glucose concentrations points to the possibility of islet cells utilizing metabolic substrates other than exogenously added glucose for lactate production. Islets of *ob/ob* mice, in particular, have considerable stores of glycogen (30). Such lactate production from endogenous metabolites explains the quite significant lactate production in the absence of added glucose in both rat and *ob/ob* mice islets.

The concentration-dependent inhibition by D-mannoheptulose of the insulin response to 20 mmol/l glucose found in this work was similar to previously reported concentration-response relationships (31,32). A similar relationship also applies to the D-mannoheptulose–induced inhibition of glucose metabolism (20 mmol/l) in rat or *ob/ob* mice islets. At its maximum effective concentration (between 5 and 20 mmol/l), the inhibitor decreased both the utilization and oxidation rates of 20 mmol/l glucose to levels 50–70% above those obtained at 3 mmol/l glucose in the absence of inhibitor. A similar concentration-response relationship for D-mannoheptulose inhibition of either glucose utilization (20 mmol/l) (31) or oxidation (22,32) has been reported before. However, a contradiction has been noted between the capac-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/47/8/1219/364628/47-8-1219.pdf by guest on 29 February 2024

ity of D-mannoheptulose to inhibit glucose utilization (31) or oxidation (22) and its lack of effect on glucose-dependent lactate production (22,31). Our study demonstrates that lactate production in the presence of either 3 or 20 mmol/l glucose is significantly inhibited by D-mannoheptulose at 5 mmol/l or higher concentrations in both rat and *ob/ob* mice islets (Table 1 and Fig. 5). This is in contrast with the higher sensitivity to D-mannoheptulose shown by the metabolism of 20 mmol/l glucose. However, D-mannoheptulose did not significantly affect the utilization and oxidation rates in the presence of 3 mmol/l glucose in either rat islets or *ob/ob* mice islets. This is compatible with a preferential inhibition of a high K_m component of islet glycolysis by D-mannoheptulose (3).

D-Mannoheptulose has been shown to behave as a competitive inhibitor of glucose phosphorylation by islet homogenates (21,33) or by the chromatographically purified glucokinase isoenzyme (23), with K_i values of 0.25 mmol/l (33) or 0.7 mmol/l (23). Moreover, in homogenates of *ob/ob* mice islets, D-mannoheptulose inhibits a high K_m but not a low K_m glucose phosphorylating activity (34). The aldoheptose competes with glucose for transmembrane transport in islet cells (32), but given the high capacity of this transport system, it probably does not significantly restrict D-mannoheptulose equilibration across the β-cell plasma membrane. The close to maximum inhibition of glucose metabolism (20 mmol/l) at 5 mmol/l D-mannoheptulose is compatible with the low K_i values reported for its inhibition of glucose phosphorylation (23,33). Because the contribution of glucokinase to the overall glucose phosphorylating activity is significant even at 3 mmol/l glucose, partly due to glucose-6-phosphate–induced inhibition of hexokinase, the finding of a significant inhibition of lactate production at both 3 and 20 mmol/l glucose can well be explained by mannoheptulose inhibition of glucokinase.

ACKNOWLEDGMENTS

This work was supported by a grant from the Spanish Fondo de Investigaciones Sanitarias de la Seguridad Social (93/0011-07) and by the Swedish Medical Research Council (12X-4756), the Swedish Diabetes Association, the Sahlberg Fund, and Umeå University. The research activity of O.A. and E.G. was partially supported by the Medical Department of Hoechst Ibérica, S.A.

REFERENCES

1. Hedeskov CJ: Mechanism of glucose-induced insulin secretion. *Physiol Rev* 60:442–509, 1980
2. Wollheim CB, Biden TJ: Signal transduction in insulin secretion: comparison between fuel stimuli and receptor agonists. *Ann N Y Acad Sci* 48:317–333, 1986
3. Newgard CB, McGarry JD: Factors in pancreatic β-cell signal transduction. *Ann Rev Biochem* 64:689–719, 1995
4. Malaisse WJ, Sener A, Herchuelz A, Hutton JC: Insulin release: the fuel hypothesis. *Metab Clin Exp* 28:373–386, 1979
5. MacDonald MJ: Elusive proximal signals of β-cells for insulin secretion. *Diabetes* 39:1461–1466, 1990
6. Hellman B, Idahl L-Å, Sehlin J, Taljedal I-B: Influence of anoxia on glucose metabolism in pancreatic islets: lack of correlation between fructose-1,6-diphosphate and apparent glycolytic flux. *Diabetologia* 11:495–500, 1975
7. Malaisse WJ: Glucose-sensing by the pancreatic β-cell: the mitochondrial part. *Int J Biochem* 24:693–701, 1992
8. Malaisse WJ, Rasschaert J, Zahner D, Sener A: Hexose metabolism in pancreatic islets: the Pasteur effect. *Diabetes Res* 7:53–58, 1988
9. Malaisse WJ, Kawazu S, Herchuelz A, Hutton JC, Somers G, Devis G, Sener A: The stimulus secretion coupling of glucose-induced insulin release: effect of lactate upon islet function. *Arch Biochem Biophys* 194:49–62, 1979
10. Jijakli H, Rasschaert J, Nadi AB, Leclercq-Meyer V, Sener A, Malaisse WJ: Relevance of lactate dehydrogenase activity to the control of oxidative glycolysis in pancreatic β-cells. *Arch Biochem Biophys* 327:260–264, 1996
11. Sekine N, Cirulli V, Regazzi R, Brown LJ, Giné E, Tamarit-Rodriguez J, Girotti M, Marie S, MacDonald MJ, Wollheim CB, Rutter GA: Low lactate dehydrogenase and high mitochondrial glycerol-phosphate dehydrogenase in pancreatic β-cells: potential role on nutrient sensing. *J Biol Chem* 269:4895–4902, 1994
12. Meglasson MD, Matschinsky FM: Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab Rev* 2:163–214, 1986
13. Erecinska M, Bryla J, Michalik M, Meglasson MD, Nelson D: Energy metabolism in islets of Langerhans. *Biochim Biophys Acta* 1101:273–295, 1992
14. Hellman B: Studies in obese-hyperglycemic mice. *Ann N Y Acad Sci* 13:541–558, 1965
15. Simonides WS, Zaremba R, Van Hardeveld C, Van der Laarse W: A non enzymatic method for the determination of picomol amounts of lactate using HPLC: its application to signal muscle fibers. *Anal Biochem* 169:268–273, 1988
16. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16:35–39, 1967
17. Hellerström C: A method for the microdissection of pancreatic islets in mammals. *Acta Endocrinol (Copenh)* 45:122–132, 1964
18. Herbert V, Lau KS, Gottlieb C, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–1384, 1965
19. Ashcroft SJH, Weerashinge CC, Bassett JM, Randle PJ: The pentose cycle and insulin release in mouse pancreatic islets. *Biochem J* 126:525–532, 1972
20. Vytasek R: A sensitive assay for the determination of DNA. *Anal Biochem* 120:243–248, 1982
21. Malaisse WJ, Lea MA, Malaisse-Lagae F: The effect of mannoheptulose on the phosphorylation of glucose and the secretion of insulin by islets of Langerhans. *Metab Clin Exp* 17:126–132, 1968
22. Ashcroft SJH, Hedeskov CJ, Randle PJ: Glucose metabolism in mouse pancreatic islets. *Biochem J* 118:143–154, 1970
23. Sener A, Malaisse WJ: Measurements of lactic acid in nanomolar amounts: reliability of such a method as an index of glycolysis in pancreatic islets. *Biochem Med* 15:34–41, 1976
24. Pace CS, Ellerman J, Hover BA, Stillings SN, Matschinsky FM: Multiple metabolic functions of glucose in rat pancreatic islets. *Diabetes* 24:476–488, 1975
25. Alcazar O, Giné E, Qiu-yue Z, Tamarít-Rodríguez J: The stimulation of insulin secretion by D-glyceraldehyde correlates with its rate of oxidation in islets cells. *Biochem J* 310:215–220, 1995
26. Zawalich WS, Matschinsky FM: Sequential analysis of the releasing and fuel function of glucose in isolated perifused pancreatic islets. *Endocrinology* 100:1–8, 1977
27. Sener A, Levy J, Malaisse WJ: The stimulus-secretion coupling of glucose-induced insulin release: does glycolysis control calcium transport in the β-cell? *Biochem J* 156:521–525, 1976
28. Malaisse WJ, Hutton JC, Kawazu S, Sener A: The stimulus-secretion coupling of glucose-induced insulin release: metabolic effects of menadione in isolated islets. *Eur J Biochem* 87:121–130, 1978
29. Ashcroft SJH, Lowry M: β-cell recognition of stereoisomers of D-glucose. *Diabetologia* 17:165–168, 1979
30. Hellman B, Idahl L-Å: Presence and mobilization of glycogen in mammalian pancreatic β-cells. *Endocrinology* 84:1–8, 1969
31. Zawalich WS, Pagliara AS, Matschinsky FM: Effects of iodoacetate, mannoheptulose and 3-O-methyl-glucose on the secretory function and metabolism of isolated pancreatic islets. *Endocrinology* 100:1276–1283, 1977
32. Hellman B, Idahl L-Å, Sehlin J, Simon E, Taljedal I-B: The pancreatic β-cell recognition of insulin secretagogues: transport of mannoheptulose and the dynamics of insulin release. *Mol Pharmacol* 8:1–7, 1972
33. Ashcroft SJH, Randle PJ: Enzymes of glucose metabolism in normal mouse pancreatic islets. *Biochem J* 119:5–15, 1968
34. Lenzen S, Tiedge M, Panten U: Glucokinase in pancreatic β-cells and its inhibition by alloxan. *Acta Endocrinol (Copenh)* 5:21–29, 1987

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/47/8/1219/364628/47-8-1219.pdf by guest on 29 February 2024