Underestimation of D-glucose utilisation as judged from the conversion of D-[3-³H]glucose to ³HOH

A. Sener, M.-H. Giroix, W. J. Malaisse

Laboratory of Experimental Medicine, Brussels Free University, Brussels, Belgium

Abstract

Aims/hypothesis. The conversion of D-[3-³H]glucose to ³HOH is currently measured to assess D-glucose utilisation. The validity of such a procedure was re-evaluated.

Methods. The conversion of D-[3-³H]glucose and D-[5-³H]glucose to ³HOH was measured in rat pancreatic islets, parotid cells and erythrocytes. The tritiation of lipids were also examined in islets exposed to D-[3-³H]glucose or D-[5-³H]glucose.

Results. In rat pancreatic islets and parotid cells, but not in rat erythrocytes, the generation of ³HOH from D-[3-³H]glucose underestimates the rate of D-glucose utilisation, this being apparently attributable to a partial escape from detritiation of [1-³H]glycerone-3-

phosphate. Such an escape phenomenon resulted in a higher tritiation of lipids in pancreatic islets exposed to D-[3-³H]glucose, rather than D-[5-³H]glucose. Its relative extent was affected by a number of environmental factors such as the cell type under consideration, the metabolic status of the animals, and the extracellular concentration of D-glucose.

Conclusion/interpretation. These findings impose a reservation on the use of D-[3-³H]glucose conversion to ³HOH as a tool to assess the utilisation of the hexose in some cell types. [Diabetologia (2002) 45: 1274–1284]

Keywords Pancreatic islets, parotid cells, erythrocytes, D-[3-³H]glucose or D-[5-³H]glucose conversion to ³HOH, D-glucose utilisation.

The generation of ³HOH from D-[3-³H]glucose is currently used to assess D-glucose utilisation [1, 2, 3, 4, 5, 6, 7]. This work documents that such a procedure could underestimate the true rate of D-glucose utilisation in some cell types. Moreover, it is shown that the relative extent of such an underestimation is affected by a number of environmental factors.

Materials and methods

D-[3-³H]glucose, D-[5-³H]glucose and D-[U-¹⁴C]glucose were purchased from NEN Life Science Products (Boston, Mass., USA). In experiments conducted in rat erythrocytes, D-[3-³H]glucose and D-[5-³H]glucose obtained from Amersham International (Aylesburg, UK) were also used.

Female Wistar rats were purchased from Iffa Credo (L'Arbresle, France). Male rats and female GK (Goto-Kakizaki) rats were obtained from our local colony. All animals were given free access to food (KM-04-k12; Pavan Service, Oud Turnhout, Belgium) and tap water up to the time of killing by decapitation. Blood was collected in heparinized vials and the plasma was separated for measurement of plasma D-glucose [8] and insulin [9] concentrations.

The methods used to isolate islets by the collagenase procedure [10] and to measure their protein [11] and insulin [10] content were described previously. Likewise, rat parotid cells prepared by the collagenase technique [12] and erythrocytes [13] were prepared as described.

Received: 28 March 2002 / Revised: 8 May 2002

Published online: 27 July 2002

© Springer-Verlag 2002

Corresponding author: Dr. W. J. Malaisse, Laboratory of Experimental Medicine, Brussels Free University, 808 Route de Lennik, 1070 Brussels, Belgium, E-mail: malaisse@ulb.ac.be

The methods used to measure the generation of radioactive CO₂ [14], H₂O [14], acidic metabolites [15] and amino acids [16] from ³H- or ¹⁴C-labelled D-glucose were also described previously.

To measure the ³H- and ¹⁴C-labelling of islet lipids, groups of 30 islets each were incubated for 120 min at 37°C in 80 µl of our usual HEPES-buffered and bicarbonate-buffered medium in the presence of D-[3-³H]glucose or D-[5-³H]glucose and D-[U-¹⁴C]glucose. After incubation, the islets were separated from the incubation medium (mixed with 100 µl of a phosphate buffer, 100 mmol/l, pH 7.4) by centrifugation through a layer (150 µl) of dibutylphthalate [13]. The bottom of the microfuge tube containing the islet pellet was cut and placed in 2 ml of a CHCl₃/CH₃OH (2/1, v/v) mixture. After adding 20 µl of rat plasma, sonification (3×10 s) and centrifugation (10 min at 4°C and 1000 g), the supernatant was removed, mixed with 0.8 ml H₂O, and again centrifuged for 10 min at 4°C. After the upper layer was removed, the extraction of lipid was completed according to a known method [17]. The procedures used in this study were approved by the local Animal Experimentation Ethics Committee.

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

Results

D-Glucose metabolism in islets from normal rats. A rise in D-glucose concentration from 2.8 to 16.7 mmol/l augmented ($p < 0.001$) the production of ¹⁴CO₂ from D-[U-¹⁴C]glucose and that of ³HOH from either D-[3-³H]glucose or D-[5-³H]glucose (Table 1). As expected from prior studies, the paired ratio between D-[U-¹⁴C]glucose oxidation and either D-[3-³H]glucose or D-[5-³H]glucose utilisation was also higher ($p < 0.001$) in islets exposed to 16.7 mmol/l D-glucose rather than 2.8 mmol/l D-glucose. Both at the low (2.8 mmol/l) and the high (16.7 mmol/l) concentration of D-glucose, the generation of ³HOH from D-[3-³H]glucose was lower ($p < 0.001$) than that from D-[5-³H]glucose. This contrasted with the fact that, as expected, the oxidation of D-[U-¹⁴C]glucose, whether at low or high concentration of the hexose, was not different in the islets exposed to either D-[3-³H]glucose or D-[5-³H]glucose.

To optimize the comparison between D-[3-³H]glucose and D-[5-³H]glucose utilisation, whether at low or high concentration of D-glucose, the paired ratio between D-[U-¹⁴C]glucose oxidation and D-[3-³H]glucose utilisation was compared to the paired ratio between D-[U-¹⁴C]glucose oxidation and D-[5-³H]glucose utilisation. Such a procedure indeed allows minimizing of the interference of individual variations in metabolic parameters, the oxidation of D-[U-¹⁴C]glucose being invariably used as reference. At 2.8 mmol/l D-glucose, the ratio between D-[3-³H]glucose and D-[5-³H]glucose utilisation (both calculated by reference to the paired value for D-[U-¹⁴C]glucose oxidation) averaged 59.9±2.0% (*d.f.*=69), as distinct

Table 1. Metabolism of D-glucose in islets from normal female rats

D-glucose	2.8 mmol/l	16.7 mmol/l
D-[U- ¹⁴ C]glucose oxidation ^a	12.2±0.4 (79)	65.0±2.3 (80)
D-[5- ³ H]glucose utilisation ^a	47.5±2.1 (39)	160.5±8.7 (39)
D-[3- ³ H]glucose utilisation ^a	32.0±1.8 (40)	110.0±5.5 (40)
¹⁴ CO ₂ / ³ HOH (D-[5- ³ H]glucose) ^b	24.8±0.8 (39)	42.6±1.0 (39)
¹⁴ CO ₂ / ³ HOH (D-[3- ³ H]glucose) ^b	40.6±1.0 (40)	60.1±1.3 (40)
D-[3- ³ H]/[5- ³ H]glucose utilisation ^c	59.9±2.0 (69) ^d	71.4±2.2 (69) ^d

^a Results expressed as pmol of D-glucose equivalent/islet per 120 min

^b Paired ratio between D-[U-¹⁴C]glucose oxidation and D-[3-³H]glucose or D-[5-³H]glucose utilisation, expressed as a percentage

^c Ratio between D-[3-³H]glucose and D-[5-³H]glucose utilisation, expressed as a percentage and computed from the ¹⁴CO₂/³HOH paired ratios

^d Degree of freedom shown in parentheses

($p < 0.001$) from 71.4±2.2% (*d.f.*=69) at 16.7 mmol/l D-glucose.

Labelling of islet lipids in islets from normal rats. After 120 min of incubation in 2.8 mmol/l and 16.7 mmol/l D-glucose, mixed with a tracer amount of D-[U-¹⁴C]glucose, the ¹⁴C-labelling of islet lipids averaged, respectively, 1.92±0.13 and 2.13±0.17 pmol of D-[U-¹⁴C]glucose equivalent per islet ($n=20$ in both cases). Whether at 2.8 or 16.7 mmol/l D-glucose, the ¹⁴C-labelling of islet lipids was, as expected, not different ($p > 0.15$ or more) in the islets concomitantly exposed to either D-[3-³H]glucose or D-[5-³H]glucose. Thus, in the latter case, the ¹⁴C-labelling averaged 104.0±4.0% ($n=20$) of the mean values recorded within the same experiment(s) and at the same hexose concentration in islets exposed to D-[3-³H]glucose (100.0±4.6%; $n=20$; $p > 0.5$). The ¹⁴C-labelling of islet lipids represented, at 16.7 mmol/l D-glucose, 110.2±5.5% ($n=20$) of that recorded within the same experiments at 2.8 mmol/l D-glucose (100.0±3.6%; $n=20$). Such a difference failed, however, to achieve statistical significance.

Likewise, the tritiation of islet lipids was not different ($p > 0.2$ or more) at 2.8 and 16.7 mmol/l D-glucose, whether in islets exposed to D-[3-³H]glucose or D-[5-³H]glucose (Table 2). To optimize the comparison between mean values, the tritiation of islet lipids was expressed, in each experiment and at each hexose concentration, relative to the mean corresponding value for their ¹⁴C-labelling. The tritiation of islet lipids by

Table 2. ¹⁴C- and ³H-labelling of lipids in islets incubated for 120 min at 2.8 or 16.7 mmol/l D-glucose in the presence of D-[U-¹⁴C]glucose and either D-[3-³H]glucose or D-[5-³H]glucose

D-glucose (mmol/l)		2.8		16.7	
Tritiated tracer	D-[3- ³ H]glucose	D-[5- ³ H]glucose	D-[3- ³ H]glucose	D-[5- ³ H]glucose	
¹⁴ C-labelling ^a	1824±101 (10)	2043±110 (10)	2161±185 (10)	2075±91 (10)	
³ H-labelling ^b	61.89±5.13 (10)	5.17±1.07 (10)	51.42±4.01 (10)	9.25±3.28 (10)	
³ H-labelling ^c	12.12±1.07 (10)	1.00±0.22 (10)	5.76±0.57 (10)	1.00±0.35 (10)	

^a Results expressed as fmol of D-glucose equivalent/islet, after normalization of the data collected within each experiment (25)

^b Results expressed as per cent of ¹⁴C-labelling, after normalization of the data collected within each experiment

^c Results expressed relative to the ³H-labelling (percent of ¹⁴C-labelling) of islets exposed, within the same experiment(s) and at the same concentration of D-glucose, to D-[5-³H]glucose

D-[3-³H]glucose largely exceeded ($p<0.001$) that by D-[5-³H]glucose, whether at 2.8 or 16.7 mmol/l D-glucose. In the islets exposed to 2.8 mmol/l of D-glucose, the tritiation of islet lipids by D-[3-³H]glucose averaged 1211.7±106.6% ($n=10$) of that found within the same experiment(s) in the case of D-[5-³H]glucose (100.0±21.8%; $n=10$). At 16.7 mmol/l D-glucose, the tritiation of islet lipids by D-[3-³H]glucose represented 576.4±57.1% ($n=10$) of that by D-[5-³H]glucose (100.0±34.9%; $n=10$). The difference in lipid tritiation between islets exposed to D-[3-³H]glucose versus D-[5-³H]glucose was thus more pronounced ($p<0.001$) at 2.8 mmol/l D-glucose than at 16.7 mmol/l D-glucose. When the results relative to the tritiation of islet lipids were expressed as the ratio between ³H- and ¹⁴C-labelling, even the difference between the data collected in islets exposed to D-[3-³H]glucose and D-[5-³H]glucose remained higher ($p<0.06$) at 2.8 mmol/l D-glucose (difference: 56.72±5.24%; $d.f.=18$) than at 16.7 mmol/l D-glucose (difference: 42.17±5.18%; $d.f.=18$).

d-Glucose metabolism in islets from GK rats. The metabolic and hormonal status of the GK rats used in this study is documented in Table 3. The female GK rats showed a higher plasma D-glucose concentration ($p<0.001$) and a lower insulinogenic index ($p<0.05$) than that found in female control rats examined within the same experiments (i.e. 9.74±0.22 mmol/l; $n=21$ and 4.53±0.34 U/mol; $n=20$). The plasma insulin concentration of the female GK rats was not different from that found in the control rats of the same sex (i.e. 281±23 pmol/l; $n=20$). Likewise, the protein content of the islets from female GK rats was not different from that of female control rats (2.33±0.25 µg per islet; $n=10$). The insulin content of the islets from female GK rats was higher ($p<0.05$ or less) than that of the control rats of the same sex, whether expressed per islet or per µg islet protein (i.e. 1.58±0.17 mU/islet and 5.20±0.80 pmol/µg; $n=10$ in both cases). Despite comparable age, the male GK rats were heavier ($p<0.001$) than the female GK rats. They also showed higher plasma D-glucose concentrations ($p<0.01$) and

Table 3. Metabolic and hormonal status of GK rats

Sex	Male rats	Female rats
Age (weeks)	19.4±0.4 (20)	20.5±0.6 (20)
Body weight (g)	351±4 (20)	235±2 (20)
Plasma D-glucose (mmol/l)	15.16±0.59 (20)	12.73±0.57 (20)
Plasma insulin (pmol/l)	508±42 (20)	287±21 (20)
Insulinogenic index (nmol/mol)	33.7±2.8 (20)	23.8±2.1 (20)
Islet protein content (µg/islet)	2.27±0.46 (10)	1.85±0.24 (10)
Islet insulin content (pmol/islet)	8.13±2.20 (10)	16.27±2.40 (10)
(pmol/µg protein)	4.00±0.80 (10)	9.27±0.73 (10)

insulin ($p<0.001$) concentrations, and a higher ($p<0.01$) insulinogenic index than the female GK rats. The insulin content of the islets from male GK rats was lower ($p<0.025$ or less) than that found in islets from female GK rats, whether expressed per islet or per µg protein (Table 3).

Whether at 2.8 mmol/l or 16.7 mmol/l D-glucose, the oxidation of D-[U-¹⁴C]glucose by islets from GK rats was not different in the islets concomitantly exposed to either D-[3-³H]glucose or D-[5-³H]glucose. At the two concentrations of D-glucose, the generation of ³HOH from D-[3-³H]glucose was lower ($p<0.001$) than that from D-[5-³H]glucose. Thus, at 2.8 and 16.7 mmol/l D-glucose, the generation of ³HOH from D-[3-³H]glucose averaged, respectively, 72.5±2.8% ($n=53$) and 76.1±3.2% ($n=52$) of the mean corresponding values for D-[5-³H]glucose utilisation recorded within the same experiments in GK rats of the same sex (i.e. 100.0±4.0%, $n=51$ and 100.0±3.1%, $n=50$). The paired ratio between D-[U-¹⁴C]glucose oxidation and D-[5-³H]glucose utilisation, which was virtually identical in male and female GK rats, increased ($p<0.001$) from 23.1±0.8% ($n=51$) to 39.1±1.1% ($n=50$) as a result of the rise in D-glucose concentration from 2.8 to 16.7 mmol/l. At the high

Table 4. Metabolism of D-glucose in islets from GK rats

D-glucose	2.8 mmol/l	16.7 mmol/l
D-[U- ¹⁴ C]glucose oxidation ^a	13.1±0.7 (105)	74.4±3.2 (106)
D-[5- ³ H]glucose utilisation ^a	67.6±7.2 (51)	192.4±20.5 (50)
D-[3- ³ H]glucose	48.6±4.9 (53)	152.9±12.5 (52)
¹⁴ CO ₂ / ³ HOH (D-[5- ³ H]glucose) ^b	23.1±0.8 (51)	39.1±1.1 (50)
¹⁴ CO ₂ / ³ HOH	31.1±1.2 (53)	47.8±1.5 (52)
D-[3- ³ H]/D-[5- ³ H] glucose utilisation ^c	75.6±2.9 (88) ^d	82.4±2.1 (86) ^d

^a Results expressed as pmol of D-glucose equivalent/islet per 120 min

^b Paired ratio between D-[U-¹⁴C]glucose oxidation and either D-[3-³H]glucose or D-[5-³H]glucose utilisation, expressed as a percentage

^c Ratio between D-[3-³H]glucose and D-[5-³H]glucose utilisation, expressed as a percentage and computed from the ¹⁴CO₂/³HOH paired ratios

^d Degrees of freedom shown in parentheses

concentration of D-glucose (16.7 mmol/l), such a paired ratio was lower ($p<0.025$) in GK rats than in the control rats, whilst this was not the case ($p>0.1$) at 2.8 mmol/l of D-glucose.

Further comparison between the production of ³HOH from D-[3-³H]glucose and D-[5-³H]glucose was achieved by reference to the paired value for D-[U-¹⁴C]glucose oxidation (see above).

In the GK rats, as in the control rats, the mean ratio between ³HOH production from D-[3-³H]glucose to D-[5-³H]glucose was lower at 2.8 mmol/l than at 16.7 mmol/l D-glucose (Table 4). However, this difference failed to achieve statistical significance ($p>0.05$). Moreover, both at low and high D-glucose concentrations, the ratios recorded in either all or only female GK rats were higher ($p<0.001$) than those computed in female control rats.

It should be underlined that the results recorded in male and female GK rats were not identical. At the low concentration of D-glucose (2.8 mmol/l), no difference ($p>0.3$) was found between male rats and female rats in terms of the ratio between ³HOH production from D-[3-³H]glucose to D-[5-³H]glucose. Thus, such a ratio averaged 72.5±4.3% ($d.f.=38$) and 77.9±3.9% ($d.f.=50$) in male and female GK rats. However, at 16.7 mmol/l D-glucose, the same ratio was lower ($p<0.001$) in male GK rats (73.1±3.3%; $d.f.=36$) than in female GK rats (89.1±2.4%; $d.f.=50$). Moreover, whilst such a ratio was not different ($p>0.9$) at low and high D-glucose concentrations in male GK rats, it was higher ($p<0.02$) at 16.7 mmol/l than at 2.8 mmol/l D-glucose in female GK rats.

Table 5. Metabolism of D-glucose (10.0 mmol/l) in rat parotid cells

D-[U- ¹⁴ C]glucose oxidation ^a	6.1±0.3 (28)
D-[5- ³ H]glucose utilisation ^a	30.1±2.0 (14)
D-[3- ³ H]glucose utilisation ^a	25.1±1.4 (14)
¹⁴ CO ₂ / ³ HOH (D-[5- ³ H]glucose) ^b	19.9±0.7 (14)
¹⁴ CO ₂ / ³ HOH (D-[3- ³ H]glucose) ^b	24.6±0.6 (14)
D-[3- ³ H]/[5- ³ H]glucose utilisation ^c	80.6±3.4 (24) ^d

^a Results expressed as pmol of D-glucose equivalent/10³ cells per 120 min

^b Paired ratio between D-[U-¹⁴C]glucose oxidation and either D-[3-³H]glucose or D-[5-³H]glucose utilisation, expressed as a percentage

^c Ratio between D-[3-³H]glucose and D-[5-³H]glucose utilisation expressed as a percentage and computed from the ¹⁴CO₂/³HOH paired ratios

^d Degree of freedom shown in parentheses

Experiments in parotid cells. A situation similar to that found in pancreatic islets was observed in parotid cells exposed to 10.0 mmol/l D-glucose (Table 5). Thus, despite a comparable rate of D-[U-¹⁴C]glucose oxidation in parotid cells concomitantly exposed to either D-[3-³H]glucose or D-[5-³H]glucose, the generation of ³HOH from D-[3-³H]glucose only represented 83.6±1.5% ($n=14$, $p<0.001$) of the mean corresponding value recorded in the same experiments in the presence of D-[5-³H]glucose (100.0±3.1%; $n=14$). When the ratio between ³HOH production from D-[3-³H]glucose and D-[5-³H]glucose was calculated by reference to the paired value for D-[U-¹⁴C]glucose oxidation (see above), it averaged 80.6±3.4% ($d.f.=24$), a value lower ($p<0.001$) than unity. The paired ratios between D-[U-¹⁴C]glucose oxidation and either D-[3-³H]glucose or D-[5-³H]glucose utilisation in parotid cells incubated in the presence of 10.0 mmol/l D-glucose were lower ($p<0.025$ or less) than those found in isolated pancreatic islets exposed to no more than 2.8 mmol/l D-glucose.

Experiments in erythrocytes. In erythrocytes exposed to 10.0 mmol/l D-glucose, the generation of ³HOH from D-[3-³H]glucose, when expressed relative to the corresponding mean value for D-[5-³H]glucose utilisation observed within the same experiments, averaged 94.2±3.5% ($n=12$) and 95.1±5.5% ($n=12$) in the case of tritiated D-glucose tracers both obtained from NEN and Amersham, respectively (Table 6). Pooling all available data, the utilisation of D-[3-³H]glucose averaged 94.7±3.2% ($n=24$) of the mean corresponding value found, within the same experiments, in the case of D-[5-³H]glucose (100.0±1.9%; $n=24$). Such a difference did not reach statistical significance ($p>0.1$).

A comparable ratio between D-[3-³H]glucose and D-[5-³H]glucose utilisation (93.6±4.1%; $d.f.=40$; $p>0.1$) was reached, when the generation of ³HOH from the

Table 6. Metabolism of D-glucose (10.0 mmol/l) in rat erythrocytes

D-[3- ³ H]glucose ^a	D-[5- ³ H]glucose ^a	Acidic [¹⁴ C]metabolites	Amino [¹⁴ C]acids	³ HOH
NEN	–	772±51 (12) ^b	9.24±0.47 (12)	793±29 (12)
–	NEN	894±58 (12)	9.79±0.43 (12)	848±40 (12)
AMERSHAM	–	951±62 (12)	10.05±0.68 (12)	655±46 (12)
–	AMERSHAM	787±40 (12)	9.86±0.42 (12)	685±22 (12)

^a Two commercial preparations of each tritiated D-glucose tracer were tested

^b All results are expressed as pmol of D-glucose equivalent/μl of blood per 120 min

two tritiated hexoses was calculated by reference to the paired value for D-[U-¹⁴C]glucose conversion to ¹⁴C-labelled acidic metabolites.

Thus, in erythrocytes, the generation of ³HOH from D-[3-³H]glucose was not lower than that from D-[5-³H]glucose, comparable results being recorded with preparations of D-[3-³H]glucose and D-[5-³H]glucose both obtained from either NEN or Amersham.

In these experiments, the generation of tritiated acidic metabolites from D-[3-³H]glucose and D-[5-³H]glucose did not exceed, respectively, 10.0±2.1 and 6.2±1.6 pmol/μl of blood per 120 min (*n*=12 in both cases), these two values not being different from one another. The net production of these tritiated acidic metabolites was thus two orders of magnitude lower than that of ¹⁴C-labelled acidic metabolites formed from D-[U-¹⁴C]glucose (Table 6). The latter generation was commensurate with the production of ³HOH from D-[5-³H]glucose, with a paired ratio between the net generation of ¹⁴C-labelled acidic metabolites and ³HOH production averaging 1.09±0.03 (*n*=24). No generation of tritiated amino acids from either D-[3-³H]glucose or D-[5-³H]glucose could be detected (data not shown).

Discussion

The results obtained in pancreatic islets prepared from female control rats document two major findings. Firstly, they indicate that the conversion of D-[3-³H]glucose to ³HOH is sizeably lower than that of D-[5-³H]glucose to ³HOH. Secondly, they show that the relative extent of such a difference is more pronounced at low (2.8 mmol/l) than at high (16.7 mmol/l) D-glucose concentrations. In parallel work, we observed that the difference between D-[3-³H]glucose and D-[5-³H]glucose conversion to ³HOH in pancreatic islets isolated from normal rats is always increased whenever the rate of glycolysis is decreased. For instance, this was documented in islets incubated in the absence of extracellular Ca²⁺ or exposed to exogenous D-glyceraldehyde. It is proposed, therefore, that part of the tritiated dihydroxyacetone phosphate generated from D-[3-³H]glucose could be converted to L-[1-³H]glycerol 3-phosphate, the latter tritiated ester being then incorporated into triglyce-

rides or phospholipids. Such an escape phenomenon is also compatible with prior work indicating enzyme-to-enzyme channelling of D-glyceraldehyde 3-phosphate in the aldolase/glyceraldehyde 3-phosphate dehydrogenase sequence [20, 21, 22].

In further support of the interpretation above, our results show that, in pancreatic islets from female control rats, the tritiation of lipids is higher in islets exposed to D-[3-³H]glucose rather than D-[5-³H]glucose. Such a difference was again more pronounced at 2.8 mmol/l than at 16.7 mmol/l D-glucose. In absolute terms, the difference between the tritiation of lipids in islets exposed to D-[3-³H]glucose versus D-[5-³H]glucose was much lower than the difference between the generation of ³HOH from the two tritiated tracers. It should be kept in mind, however, that several tritiated metabolites (e.g. tritiated dihydroxyacetone phosphate and L-glycerol 3-phosphate) were not measured in these experiments. Moreover, the tritiation and ¹⁴C-labelling of islet lipids is likely to be affected by their continuous turnover.

The results recorded in GK rats affords three essential pieces of information. Firstly, they confirm that, at a high but not at a low D-glucose concentration, the paired ratio between D-[U-¹⁴C]glucose oxidation and D-[5-³H]glucose utilisation is lower in islets from GK rats than in those from the control rats [18, 19]. Secondly, they show that, at 2.8 mmol/l D-glucose, the ratio between D-[3-³H]glucose and D-[5-³H]glucose conversion to ³HOH is higher (*p*<0.005) in both male GK rats and female GK rats than in female control rats. This coincides with an abnormally high basal rate of insulin release in the islets from GK rats [19]. Both phenomena could conceivably be attributed to the accumulation of glycogen in the beta cells of the hyperglycaemic GK rats and, hence, to the stimulation of glycogenolysis, with a subsequent increase in glycolytic flux, in the islets of GK rats exposed to low concentrations of D-glucose [23]. The ratio between D-[3-³H]glucose and D-[5-³H]glucose conversion to ³HOH does increase whenever the rate of glycolysis is itself increased, e.g. as a result of a rise in extracellular D-glucose concentration. Thirdly, the results indicate that, in male GK rats, with higher plasma D-glucose and insulin concentrations than in female GK rats, no further increase in the ratio between D-[3-³H]glucose and D-[5-³H]glucose conversion to ³HOH is observed

in response to a rise in hexose concentration from 2.8 to 16.7 mmol/l, at variance with the situation found in either the control rats or the GK female rats. It would seem, therefore, that in the more severely hyperglycaemic male GK rats, the ratio between D-[3-³H]glucose and D-[5-³H]glucose conversion to ³HOH could already have reached its close-to-maximal value in islets exposed to no more than 2.8 mmol/l D-glucose.

In parotid cells, the generation of ³HOH from D-[3-³H]glucose was also lower than that from D-[5-³H]glucose. The ratio between D-[3-³H]glucose and D-[5-³H]glucose conversion to ³HOH was higher ($p < 0.05$ or less) in parotid cells than in islets all prepared from female control rats. Thus, such a ratio is not identical in all cell types.

This concept is borne out by the findings made in erythrocytes. In the latter cells, no significant difference between the generation of ³HOH from D-[3-³H]glucose and D-[5-³H]glucose was observed, confirming a prior observation [24]. Such a ratio was higher ($p < 0.05$) in erythrocytes than in parotid cells both exposed to 10.0 mmol/l D-glucose. The absence of a difference between D-[3-³H]glucose and D-[5-³H]glucose conversion to ³HOH in erythrocytes could well be due to a low turnover of phospholipids in these ageing cells. The results obtained in erythrocytes are reassuring in two respects. Firstly, they indicate that a lower generation of ³HOH from D-[3-³H]glucose than from D-[5-³H]glucose, whenever observed, is not attributable to isotopic discrimination. Secondly, they show that virtually identical results were obtained with two distinct commercial preparations of both D-[3-³H]glucose and D-[5-³H]glucose.

It has been proposed that, in the isolated working heart, ³HOH production from D-[5-³H]glucose could overestimate glycolytic flux, possibly through generation of ³HOH in the L-type pentose phosphate pathway and/or through transaldolase exchange [26]. In this respect, it should also be kept in mind that the ³H from D-[3-³H]glucose is lost as NADP³H in the oxidative portion of the pentose phosphate pathway. Although this and more recent information [27] should not be ignored, several findings argue against a major role for these processes in the difference found here between D-[3-³H]glucose and D-[5-³H]glucose conversion to ³HOH in isolated pancreatic islets. Firstly, at variance with the situation found in the isolated working heart, the generation of ³HOH from D-[5-³H]glucose does not progressively increase during incubation of isolated rat pancreatic islets. On the contrary, in the islets, the rate of ³HOH generation from D-[5-³H]glucose is higher during the first 15 min of incubation than thereafter [28]. Secondly, the flux through the pentose phosphate pathway only accounts for a minor fraction of D-glucose metabolism in pancreatic islets [19, 29, 30]. It should be mentioned that the proposed partial escape of glycero-3-phosphate from glycoly-

sis would be expected to minimize the difference between D-[1-¹⁴C]glucose and D-[6-¹⁴C]glucose oxidation. Thirdly, the participation of the pentose phosphate pathway in the total metabolism of D-glucose is not significantly different in islets prepared from control rats and GK rats [19]; this is in contrast with our findings concerning the ratio between D-[3-³H]glucose and D-[5-³H]glucose conversion to ³HOH in the islets from the control versus GK rats.

In conclusion, this study documents that the generation of ³HOH from D-[3-³H]glucose underestimates the true rate of D-glucose utilisation in several cell types. Moreover, it shows that the relative extent of such an underestimation is affected by a number of factors such as the cell type under consideration, the concentration of extracellular D-glucose, the metabolic status of the animals (control rats vs diabetic rats) and the severity of hyperglycaemia in diabetic animals (male GK rats vs female GK rats). In our opinion, this information imposes reservation on the use of D-[3-³H]glucose conversion to ³HOH as a tool to assess the utilisation of the hexose in some cell types.

Acknowledgements. This work was supported by grants from the Belgian Foundation for Scientific Medical Research (3.4513.94 and 3.4567.97), the National Institute for Health and Medical Research (INSERM, France) and the French Community of Belgium (CFB, Belgium) (Project 1999/2000). We are indebted to J. Schoonheydt and M. Urbain for technical assistance, and C. Demesmaeker for secretarial help.

References

1. Katz J, Golden S, Dunn A, Chenoweth M (1976) Estimation of glucose turnover in rats in vivo with tritium labeled glucoses. *Hoppe-Seyler's Z Physiol Chem* 357:1387–1394
2. Katz J, Rognstad R (1976) Futile cycles in the metabolism of glucose. *Curr Top Cell Regul* 10:237–289
3. Hue L (1981) The role of futile cycles in the regulation of carbohydrate metabolism in the liver. In: Meister A (ed) *Advances in enzymology*, vol 52. Wiley, New York, pp 247–231
4. Bell PM, Firth RG, Rizza RA (1986) Effects of hyperglycemia on glucose production and utilization in humans. Measurement with [²H]-, [³H]-, and [⁶14C]glucose. *Diabetes* 35:642–648
5. Rossetti L, Giaccari A (1990) Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. A dose-response euglycemic clamp study in normal and diabetic rats. *J Clin Invest* 85:1785–1792
6. Rossetti L, Lee Y-T, Ruiz J, Aldridge SC, Shamon H, Boden G (1993) Quantitation of glycolysis and skeletal muscle glycogen synthesis in humans. *Am J Physiol* 265:E761–E769
7. Féry F, Devière J, Balasse EO (2001) Metabolic handling of intraduodenal vs. intravenous glucose in humans. *Am J Physiol* 281:E261–E268
8. Bergmeyer HU, Berndt E (1974) Determination with glucose oxidase and peroxidase. In: Bergmeyer HU (ed) *Methods of enzymatic analysis*. Academic Press, New York, pp 1205–1215

9. Leclercq-Meyer V, Marchand J, Woussen-Colle M-C, Giroix M-H, Malaisse WJ (1985) Multiple effects of leucine on glucagon, insulin and somatostatin secretion from the perfused rat pancreas. *Endocrinology* 116:1168–1174
10. Malaisse-Lagae F, Malaisse WJ (1984) Insulin release by pancreatic islets. In: Lerner J, Pohl SL (eds) *Methods in diabetes research*, vol 1. Wiley, New York, pp 147–152
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 153:265–275
12. Mangos JA, McSherry NR, Butcher F, Irvin K, Barber T (1975) Dispersed parotid cells. I. Morphological and functional characterization. *Am J Physiol* 229:553–559
13. Ramirez R, Courtois P, Ladrière L, Kadiata MM, Sener A, Malaisse WJ (2001) Uptake of D-mannoheptulose by rat erythrocytes, hepatocytes and parotid cells. *Int J Mol Med* 8:37–42
14. Malaisse WJ, Sener A (1988) Hexose metabolism in pancreatic islets. Feedback control of D-glucose oxidation by functional events. *Biochim Biophys Acta* 971:246–254
15. Malaisse WJ, Giroix M-H, Malaisse-Lagae F, Sener A (1986) 3-O-methyl-D-glucose transport in tumoral insulin-producing cells. *Am J Physiol* 251:C841–C846
16. Hutton JC, Sener A, Malaisse WJ (1979) The metabolism of 4-methyl-2-oxopentanoate in rat pancreatic islets. *Biochem J* 184:291–301
17. Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509
18. Giroix M-H, Sener A, Portha B, Malaisse WJ (1993) Preferential alteration of oxidative relative to total glycolysis in pancreatic islets of two rat models of inherited or acquired Type II (non-insulin-dependent) diabetes mellitus. *Diabetologia* 36:305–309
19. Giroix M-H, Scruel O, Ladrière L, Sener A, Portha B, Malaisse WJ (1999) Metabolic and secretory interactions between D-glucose and D-fructose in islets from GK rats. *Endocrinology* 140:5556–5565
20. Ovádi J, Keleti T (1978) Kinetic evidence for interaction between aldolase and D-glyceraldehyde-3-phosphate dehydrogenase. *Eur J Biochem* 85:157–161
21. Vértessy B, Ovádi O (1987) A simple approach to detect active-site-directed enzyme-enzyme interactions: the aldolase/glycerol-phosphate-dehydrogenase enzyme system. *Eur J Biochem* 164:655–659
22. Kvassman J, Petersson G, Ryde-Petersson U (1988) Mechanisms of glyceraldehyde-3-phosphate transfer from aldolase to glyceraldehyde-3-phosphate dehydrogenase. *Eur J Biochem* 172:427–431
23. Malaisse WJ, Marynissen G, Sener A (1992) Possible role of glycogen accumulation in B-cell glucotoxicity. *Metabolism* 41:814–819
24. Manuel y Keenoy B, Malaisse-Lagae F, Malaisse WJ (1991) Metabolism of tritiated D-glucose in rat erythrocytes. *Metabolism* 40:978–985
25. Jijakli H, Ulusoy S, Malaisse WJ (1996) Dissociation between the potency and reversibility of the insulinotropic action of two meglitinide analogues. *Pharmacol Res* 34:105–108
26. Goodwin GW, Cohen DM, Taegtmeier H (2001) [5-³H]-glucose overestimates glycolytic flux in isolated working rat heart: role of the pentose phosphate pathway. *Am J Physiol* 280:E502–E508
27. Leong HS, Grist M, Parsons H et al. (2002) Accelerated rates of glycolysis in the hypertrophied heart: are they a methodological artefact? *Am J Physiol* 282:E1039–E1045
28. Sener A, Malaisse-Lagae F, Malaisse WJ (1993) Hexose metabolism in pancreatic islets: time-course of the oxidative response to glucose. *Biochim Biophys Acta* 1177: 54–60
29. Malaisse WJ, Sener A, Levy J, Herchuelz A (1976) The stimulus-secretion coupling of glucose-induced insulin release. XXII. Qualitative and quantitative aspects of glycolysis in isolated islets. *Acta Diabetol Lat* 13:202–215
30. Giroix M-H, Sener A, Malaisse WJ (1985) Pentose cycle pathway in normal and tumoral islet cells. *FEBS Lett* 185:1–3