Quantification of glucose cycling and the extent of equilibration of glucose 6-phosphate with fructose 6-phosphate in islets from *ob/ob* mice

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Pancreatic islets from fed and fasted obese hyperglycaemic (*ob/ob*) mice were incubated with [1-¹⁴C]glucose at 5.5 mM and 16.7 mM, [1-¹⁴C]mannose at 16.7 mM, and ³H₂O. Yields of ¹⁴CO₂ and ¹⁴C-labelled lactate, and amounts of ¹⁴C from [1-¹⁴C]mannose incorporated into glucose and of ³H bound to C-2 of glucose, were measured. Glucose utilization was determined from yields of ³H₂O from [5-³H]glucose. From the results using ¹⁴C, 32–43 % of the hexoses phosphorylated to hexose 6-phosphate were estimated to have been dephosphorylated, i.e. cycled. Estimates of hexose cycling from ³H incorporation into glucose were similar. Differences in the ratios of ¹⁴C yields in CO₂ and lactate indicated incomplete isotopic equilibration between glucose 6-phosphate and fructose 6-phosphate, making the estimates of cycling semi-quantitative. In the fasted state, a larger percentage of the hexose utilized went to lactate than in the fed state. Thus conversion of mannose into glucose in islets indicates the occurrence of glucose cycling in islets. Yields of ¹⁴C from [1-¹⁴C]mannose, compared with from [1-¹⁴C]glucose, provide an approach for quantifying the extent of this cycling. These data provide further evidence for extensive glucose cycling occurring in *ob/ob* islets in both the fed and the fasted state.

INTRODUCTION

Glucose-6-phosphatase (EC 3.1.3.9) activity has been demonstrated in endoplasmic reticulum from rodent pancreatic islets [1–3]. We obtained an estimate of the extent of phosphatase function in islets from fasted obese hyperglycaemic (*ob/ob*) mice by determining the amount of glucose 6-phosphate (glucose-6-*P*) hydrolysed to glucose during the utilization of glucose by the islets [4]. Glucose → glucose-6-*P* → glucose, i.e. glucose cycling, was measured from the incorporation of ³H at position C-2 of glucose on incubating the islets with ³H₂O and unlabelled glucose. We estimated that about 40 % of the glucose phosphorylated by the islets at a glucose concentration of 5.5 mM was subsequently dephosphorylated. We next incubated islets from fed *ob/ob* mice and their lean littermates [5]. The amount of cycling in islets from the lean mice, again measured by ³H incorporation, was estimated to be only about 3.5 % at both 5.5 and 16.7 mM-glucose, compared with an estimated 18 % at 5.5 mM-glucose and 30 % at 16.7 mM-glucose in islets from the *ob/ob* mice. Using the same method, the amount of glucose phosphorylated by islets of neonatally streptozotocin-induced diabetic rats that was then dephosphorylated (at 16.7 mM-glucose) was estimated to be 16 %, about four times the percentage phosphorylated by islets from normal rats [6].

These estimates depend on the assumption that, before each molecule of glucose-6-*P* is hydrolysed to glucose, a hydrogen atom from the medium equilibrates with the hydrogen bound to C-2 of the glucose-6-*P* because of rapid equilibration between glucose-6-*P* and fructose-6-*P*. There is support for this being a good approximation. Thus, phosphohexose isomerase activity is relatively high in islets [7]. Also, only about 25 % of the ³H of [2-³H]glucose is retained in glucose cycling in the liver under various conditions [8]. Further, of the theoretical maximum of one atom of ³H from ³H₂O bound to C-2 during gluconeogenesis and in the conversion of glucose into liver glycogen, 0.77–0.92 atoms are

actually incorporated [9]. Because isotopic equilibration is incomplete, the extent of cycling is underestimated by using ³H₂O.

Because of the possible importance of glucose cycling in regulating insulin secretion by β -cells and to confirm the remarkable extent of cycling in the islets from the *ob/ob* mice, determined by ³H incorporation, we have developed and applied another method for estimating cycling in islets. An estimate of the quantity of glucose-6-*P* converted into glucose is obtained from measuring the quantity of ¹⁴C-labelled mannose that is converted into ¹⁴C-labelled glucose. Demonstration that mannose is converted into glucose by islets has added significance, since the failure to observe such a conversion (although under conditions other than we have used here) has resulted in the conclusion that glucose-6-phosphatase, while present in islets, did not play a role in insulin secretion [10,11].

To quantify cycling using mannose, rapid equilibration is assumed between fructose-6-*P* formed from the mannose [7], i.e. mannose → mannose-6-*P* → fructose-6-*P*, and glucose-6-*P*. There are conflicting reports on the extent of this equilibration in isolated islets incubated with mannose [11–13]. While the use of ³H₂O to measure cycling is also dependent on the extent of this equilibration, ¹⁴C is less likely than ³H to result in an underestimation of cycling because of an isotope effect. By incubating islets simultaneously with ¹⁴C-labelled mannose and ³H₂O, we have compared estimates of glucose cycling using the two methods. By comparing the fates of glucose carbon atoms with those of mannose carbon atoms, we obtained a measure of the extent of isotopic equilibration of ¹⁴C-labelled fructose-6-*P* with glucose-6-*P*.

MATERIALS AND METHODS

Islets from *ob/ob* mice

ob/ob mice, weighing 46–71 g, and their lean littermates, weighing 32–38 g, were killed by decapitation. The pancreata

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were cleaned of fat tissue, washed in Hanks' solution and minced, and the mince was digested at 37 °C in Hanks' solution containing collagenase [14]. The suspension was diluted with additional cold solution and allowed to settle, and the sediment was washed twice more with cold solution. Islets from the sediment were then collected using a glass pipette under stereo microscopy. Blood glucose concentrations at the time of killing were determined using glucose oxidase (model 23A Glucose Analyser; Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.).

Radiochemicals

[1-¹⁴C]Glucose was purchased from New England Nuclear. By h.p.l.c. on an Aminex HPX-87P column (Bio-Rad Laboratories) with water as solvent at 80 °C, it gave a single peak of ¹⁴C having the mobility of glucose [15]. [1-¹⁴C]Mannose was purchased from Amersham Research Products and Research Products International. Each lot was subjected to h.p.l.c. on the HPX-87P system. Glucose is well separated from mannose in this system, and a peak of ¹⁴C with the mobility of mannose was isolated and used. [5-³H]Glucose and ³H₂O were also purchased from New England Nuclear.

Incubations with [1-¹⁴C]hexoses

Islets from the *ob/ob* mice, fed or fasted for 24 h, were incubated in four media. One contained 5.5 mM-glucose, 16.7 mM-mannose, 0.5 % BSA (fraction V) and 0.5–1.1 μCi of [1-¹⁴C]glucose in Krebs bicarbonate buffer, pH 7.4. The second was identical, except it contained 0.3–0.8 μCi of [1-¹⁴C]mannose and no [1-¹⁴C]glucose. The third and fourth media were identical with the first and second respectively, except that in each the glucose concentration was 16.7 mM.

To each of four 0.6 ml incubation flasks, 50 islets were added in 100 μl of one of the four media described (one medium in each flask). Each flask with its contents was suspended in a glass scintillation vial and gassed with O₂/CO₂ (19:1), and the vial was sealed. After 3 h of incubation at 37 °C, 100 μl of 10 % HClO₄ was injected into the flask and 250 μl of Hyamine (New England Nuclear) was injected into the vial to absorb CO₂. Parallel incubations were performed without islets.

CO₂ was collected overnight. The flask with its acidified contents was removed from the vial, and Aquasure (10 ml; New England Nuclear) was added to the vial, which was then assayed for radioactivity in a liquid scintillation spectrometer. The ¹⁴CO₂ formed by the islets in each medium was defined as the difference in the ¹⁴C in CO₂ in that particular medium incubated in the presence and in the absence of islets. The amount of ¹⁴CO₂ formed (pmol) was calculated by dividing the radioactivity (d.p.m.) in the CO₂ by the specific radioactivity (d.p.m./pmol) of the ¹⁴C-labelled hexose in the medium. To each acidified incubate that contained [1-¹⁴C]mannose, 2.5 mg of glucose was added as carrier, and then the incubate was neutralized with KOH and KClO₄, which were precipitated and removed by centrifugation (3000 g, 10 min). The supernatant was passed through a mixed-bed ion-exchange resin (MB-3; BDH, Poole, Dorset, U.K.) and the effluent was freeze-dried.

A solution of the freeze-dried sample in water was chromatographed using the HPX-87P system. The fraction eluting with the mobility of glucose was assayed for ¹⁴C and for glucose content using glucose oxidase. When this fraction was incubated with glucose oxidase and the resulting reaction mixture was deionized, concentrated, and the concentrate chromatographed, there was no longer any ¹⁴C present with the mobility of glucose. Values of d.p.m./mg of glucose were calculated from the ¹⁴C radioactivity and the glucose content in the fraction. The radioactivity in-

corporated into glucose was taken as the difference in ¹⁴C with the mobility of glucose in the presence and in the absence of islets.

The amounts of glucose formed from mannose (pmol) were calculated by dividing the radioactivity (d.p.m.) incorporated into glucose in the presence of islets less that incorporated in their absence by the specific radioactivity of the [1-¹⁴C]mannose (d.p.m./pmol). These values were multiplied by 2.6 when the glucose concentration in the incubate was 5.5 mM, i.e. the 2.5 mg of glucose added to the 0.1 mg of glucose in the incubate at 5.5 mM and by 2.8 when the glucose concentration was 16.7 mM, i.e. the 2.5 mg of glucose added to the 0.3 mg at 16.7 mM.

Islets from the 24 h-fasted *ob/ob* mice were incubated with [1-¹⁴C]mannose (16.7 mM) and glucose (5.5 mM), as just described, except that islets were incubated for 90 min as well as for 3 h, and only the amount of ¹⁴C incorporated into glucose was determined. Islets from the lean littermates, fasted for 24 h, were also incubated for 3 h with [1-¹⁴C]mannose (16.7 mM) and glucose (5.5 mM), and the incorporation of ¹⁴C into glucose was determined.

Incubations with [1-¹⁴C]hexoses and ³H₂O

Without isolation of lactate. Islets from fed mice were incubated as described above for [1-¹⁴C]hexoses alone with glucose at 5.5 mM, except that 2.5 mCi of ³H₂O was added to each incubate. Because Hyamine will absorb ³H₂O, instead of using this substance to absorb the ¹⁴CO₂ released from the acidified incubate, 2 ml of CO₂-free 1 M-NaOH was added to the vial. After collecting the ¹⁴CO₂ overnight, BaCl₂ was added to precipitate the ¹⁴CO₂ as Ba¹⁴CO₃. The Ba¹⁴CO₃ was collected by filtration, washed with CO₂-free water and dried to remove any ³H₂O absorbed by the NaOH solution and adhering to the Ba¹⁴CO₃. The Ba¹⁴CO₃ was placed at the bottom of a flask capped with a rubber stopper from which a scintillation vial was suspended. Hyamine was injected through the stopper into the vial and sulphuric acid was injected on to the Ba¹⁴CO₃. After the ¹⁴CO₂ had been absorbed by the Hyamine, Aquasure was added to the vial and its contents were assayed for ¹⁴C.

Before the freeze-dried sample from the perchlorate-treated acidified incubate was chromatographed, it was dissolved in 2 ml of water and the water was evaporated. Otherwise a peak of ³H₂O followed the glucose peak. A portion of glucose from each incubate with islets, and all of the glucose from each incubate without islets, when [1-¹⁴C]mannose was in the incubate, was oxidized to CO₂ and H₂O using a Model 306 Packard sample oxidizer (Packard Instrument Co., LaGrange, IL, U.S.A.), and the samples were assayed for radioactivity. The amount of [1-¹⁴C]mannose converted into glucose was calculated as described above for when no ³H₂O was present in the incubate. The amount of ³H incorporated into glucose was calculated by dividing the ³H (d.p.m.) incorporated into glucose when islets were present by 0.5, the d.p.m./patom of hydrogen in the incubate (i.e. there are 5.5 × 10⁹ pmol of H₂O and therefore 11.0 × 10⁹ patoms of hydrogen in 0.1 ml of water, the volume of the incubate, and there are 5.5 × 10⁹ d.p.m. in 2.5 mCi of ³H₂O added to the incubate). There was negligible ³H in glucose in the absence of islets.

To determine the amount of ³H bound to C-2 of glucose, an osazone was prepared from another portion of the glucose from the incubate with islets [4,5,8]. The osazone was also oxidized to CO₂ and H₂O, which were assayed for radioactivity. The fraction of ³H in glucose other than at C-2 was then (³H/¹⁴C in osazone)/(³H/¹⁴C in glucose), and the fraction at position C-2 was 1 – [(³H/¹⁴C in osazone)/(³H/¹⁴C in glucose)].

With isolation of lactate. Islets from fed and fasted mice were incubated at glucose concentrations of 5.5 and 16.7 mM as

Table 1. Dietary state, body weight, blood glucose concentrations, estimates of conversion of [1-¹⁴C]glucose and [1-¹⁴C]mannose into CO₂ and glucose, hexose utilization and cycling

Conversions and utilizations are in pmol/h per islet. Means $\pm$ S.E.M. are recorded for the numbers of animals in parentheses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus 5.5 mM-glucose, and † $P < 0.001$ versus fed. For body weight and blood glucose values, Student's *t* test for unpaired observations was performed. For other parameters, the *t* test for paired observations was used.

	Fed		Fasted	
Weight (g)	64 $\pm$ 2 (13)		59 $\pm$ 7 (13)	
Blood glucose (mM)	14 $\pm$ 1 (13)		9 $\pm$ 1 (13)†	
Glucose in incubate (mM)	5.5	16.7	5.5	16.7
[1- ¹⁴ C]Glucose $\rightarrow$ CO ₂	56 $\pm$ 17 (5)	101 $\pm$ 17 (5)**	42 $\pm$ 10 (5)*	118 $\pm$ 36 (5)*
[1- ¹⁴ C]Mannose $\rightarrow$ CO ₂	91 $\pm$ 16 (5)	84 $\pm$ 25 (5)	73 $\pm$ 20 (5)	65 $\pm$ 13 (5)
[1- ¹⁴ C]Mannose $\rightarrow$ glucose	138 $\pm$ 22 (5)	141 $\pm$ 23 (5)	94 $\pm$ 16 (5)	119 $\pm$ 12 (5)
[1- ¹⁴ C]Glucose $\rightarrow$ glucose	104 $\pm$ 25	217 $\pm$ 66*	54 $\pm$ 10	212 $\pm$ 49*
Hexose $\rightarrow$ glucose	227 $\pm$ 36	358 $\pm$ 87	148 $\pm$ 24	331 $\pm$ 54**
Glucose utilization	116 $\pm$ 14 (6)	278 $\pm$ 21 (6)***	116 $\pm$ 23 (8)	323 $\pm$ 70 (16)**
Mannose utilization	184	223	201	178
Glucose + mannose utilization	297	490	317	500
Hexose cycling (%)	43	42	32	40

described, except that 2.5 mg of glucose was added to the acidified incubates containing [1-¹⁴C]glucose as well as [1-¹⁴C]-mannose. L-Lactic acid (2.5 mg) was also added to each incubate.

The acidified incubate after the collection of CO₂ was neutralized with KOH and the KClO₄ was removed as before, but then the supernatant was freeze-dried and the residue was dissolved in water. This solution was acidified with H₂SO₄ and continuously extracted with ether. The solution after ether extraction was deionized by passage through the mixed-bed resin. Glucose was isolated from the effluent and oxidized to CO₂ and H₂O, which were assayed for radioactivity. The amounts (pmol) of ¹⁴C incorporated into glucose in the incubate containing [1-¹⁴C]-mannose and of ³H incorporated into glucose in the incubates containing [1-¹⁴C]mannose and [1-¹⁴C]glucose were then calculated as described above. There was negligible crossover into ³H₂O of ¹⁴C on oxidation of the samples from the incubates containing [1-¹⁴C]glucose. An indication of the adequacy of the isolation procedure was the observation that the amount of ³H incorporated into glucose in the incubate containing [1-¹⁴C]-mannose was essentially the same as the amount in the similar incubate containing [1-¹⁴C]glucose.

The ether extract was neutralized with NaOH and evaporated to dryness. The sodium-lactate-containing residue was subjected to h.p.l.c. using an HPX-87H column. The lactate peak, identified by enzymic assay using lactic acid dehydrogenase [16], was collected, acidified and extracted continuously with ether. This ether extract was also neutralized and evaporated to dryness, and the residue was chromatographed using the HPX-87H system and the lactate peak was collected and assayed for lactate content. An aliquot of this peak was oxidized to CO₂ and H₂O, which were assayed for radioactivity. The amounts (pmol) of ¹⁴C and ³H incorporated into lactate by the islets were calculated by multiplying the amounts of ¹⁴C and ³H/mg of lactate by 2.5, the amount of lactate that had been added as carrier, and dividing (1) by the specific radioactivity (d.p.m./pmol) of the [1-¹⁴C]hexose in the incubate, and (2) by 0.5, the specific radioactivity of the ³H in the ³H₂O in the incubate.

In the absence of islets, less than one-fifth as much ¹⁴C and one-fiftieth as much ³H was incorporated into lactate compared with the situation in the presence of islets. Correction was made for these amounts. A measure of the adequacy of the isolation procedure was the observation that the amount of ³H incor-

porated into lactate in the incubate containing [1-¹⁴C]mannose was essentially the same as the amount incorporated into lactate in the similar incubate containing [1-¹⁴C]glucose.

Measurement of glucose utilization

Islets were incubated in an identical manner to that with ¹⁴C-labelled mannose and glucose, except that only glucose was labelled, and with [5-³H]glucose. Utilization of glucose was calculated from the yields of ³H₂O [17].

Estimation of mannose utilization, glucose-6-*P* hydrolysis and glucose cycling

Having calculated (in pmol/h per islet) (1) glucose utilized, (2) ¹⁴C from [1-¹⁴C]glucose and [1-¹⁴C]mannose oxidized to ¹⁴CO₂ and incorporated into lactate, and (3) [1-¹⁴C]mannose converted into glucose, the following additional calculations were made:

$$\text{Mannose utilization} = \frac{[\text{1-}^{14}\text{C}]\text{mannose} \rightarrow ^{14}\text{CO}_2}{[\text{1-}^{14}\text{C}]\text{glucose} \rightarrow ^{14}\text{CO}_2} \times \text{glucose utilization} \quad (1)$$

$$\text{Glucose} \rightarrow \text{glucose} = \frac{[\text{1-}^{14}\text{C}]\text{glucose} \rightarrow ^{14}\text{CO}_2}{[\text{1-}^{14}\text{C}]\text{mannose} \rightarrow ^{14}\text{CO}_2} \times [\text{1-}^{14}\text{C}]\text{mannose} \rightarrow \text{glucose} \quad (2)$$

$$\text{Glucose} \rightarrow \text{glucose} = \frac{[\text{1-}^{14}\text{C}]\text{glucose} \rightarrow \text{lactate}}{[\text{1-}^{14}\text{C}]\text{mannose} \rightarrow \text{lactate}} \times [\text{1-}^{14}\text{C}]\text{mannose} \rightarrow \text{glucose} \quad (3)$$

The total amount of hexose phosphorylated is then the sum of glucose utilization and mannose utilization. The quantity of glucose-6-*P* converted into glucose is the sum of the hexoses, i.e. glucose and mannose, converted into glucose. Then:

$$\text{Hexose cycling (\%)} = \frac{\text{hexoses} \rightarrow \text{glucose}}{\text{hexoses} \rightarrow \text{glucose} + (\text{glucose utilization} + \text{mannose utilization})} \times 100 \quad (4)$$

RESULTS

Yields in the absence and in the presence of islets

In the incubations with [^{14}C]hexoses, between 0.2% and 0.6% of the ^{14}C added as [$1\text{-}^{14}\text{C}$]mannose, and between 0.5% and 0.9% of the ^{14}C added as [$1\text{-}^{14}\text{C}$]glucose, was collected as $^{14}\text{CO}_2$ in the absence of islets. The yield of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]mannose in the presence of islets was on average 3.2 times (range 1.6–7.4 times) greater than that in the absence of islets. The yield from [$1\text{-}^{14}\text{C}$]glucose was on average 2.4 times (range 1.5–4.3 times) greater. In the absence of islets, the amount of ^{14}C with the mobility of glucose averaged 0.7% (range 0.3–1.5%) of the ^{14}C added as [$1\text{-}^{14}\text{C}$]mannose. When islets were present, the amount of ^{14}C with the mobility of glucose averaged 3.3-fold more (range 1.6–5.7-fold) than that in the absence of islets. With the ^{14}C preparations used in the incubates with $^3\text{H}_2\text{O}$, only 0.2–0.3% of the ^{14}C added as [$1\text{-}^{14}\text{C}$]mannose and 0.1–0.2% of the ^{14}C added as [$1\text{-}^{14}\text{C}$]glucose was collected as $^{14}\text{CO}_2$ in the absence of islets, and only 0.1% of the [$1\text{-}^{14}\text{C}$]mannose was recovered as ^{14}C -labelled glucose in the absence of islets. Ratios of ^{14}C yield in glucose from [$1\text{-}^{14}\text{C}$]mannose in the presence of islets/ ^{14}C in glucose in the absence of islets averaged 10.2 (range 7.2–13.3).

Incubations with [^{14}C]hexoses

Results from the incubations of islets from fed and fasted *ob/ob* mice with [^{14}C]hexoses and the measurements of glucose utilization are recorded in Table 1. The mean body weights of the two groups of mice were similar. Blood glucose concentrations were higher at the time of death in the fed mice. $^{14}\text{CO}_2$ yields from [$1\text{-}^{14}\text{C}$]glucose at a concentration of 5.5 mM-glucose were somewhat less and at 16.7 mM-glucose somewhat more than from [$1\text{-}^{14}\text{C}$]mannose at 16.7 mM. There was no difference between fed and fasted mice. There was 2–4 times as much glucose cycling, i.e. glucose phosphorylated to glucose-6-*P* and then hydrolysed back to glucose, at 16.7 mM-glucose compared with at 5.5 mM-glucose (calculated from eqn. 2). The amount of hexose converted into glucose was about twice as much at a concentration of 16.7 mM as at 5.5 mM. Glucose utilization at 16.7 mM-glucose was more than twice as high as that at 5.5 mM-glucose, while mannose utilization, calculated using eqn. (1), was similar whether glucose was at 5.5 or 16.7 mM. Again, there was no difference between the fed and fasted mice. Hexose utilization was about two-thirds more at 16.7 mM-glucose than at 5.5 mM-glucose. Glucose cycling, calculated using eqn. (4), ranged from 32 to 43%.

In three incubations in which islets from fasted mice were incubated for 90 min or 3 h with [$1\text{-}^{14}\text{C}$]mannose, incorporation of ^{14}C into glucose at 3 h was about twice that at 90 min (ratios of incorporation at 3 h to that at 90 min in the three incubations were 2.3, 1.9 and 1.7). Blood glucose concentrations ranged from 4.6 to 7.2 mM. Incorporation of ^{14}C into glucose in the absence of islets at both 90 min and 3 h was approx. one-quarter of the incorporation at 3 h in the presence of islets. In three incubations of islets from the lean mice with [$1\text{-}^{14}\text{C}$]mannose and unlabelled glucose, incorporation into glucose was the same as in incubations without islets. Blood glucose concentrations ranged from 4.8 to 6.1 mM.

Incubations with [^{14}C]hexoses and $^3\text{H}_2\text{O}$

Results of the incubations with $^3\text{H}_2\text{O}$ plus [^{14}C]hexoses are presented in Table 2. The ratio of the yield of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]glucose to that from [$1\text{-}^{14}\text{C}$]mannose at 16.7 mM-glucose appeared to be higher than at 5.5 mM-glucose (column *a*). In the fasted state, the yield of ^{14}C -labelled lactate was less from [$1\text{-}^{14}\text{C}$]glucose than from [$1\text{-}^{14}\text{C}$]mannose at 5.5 mM-glucose, but

Table 2. Results of incubations of islets from fed and fasted mice with [$1\text{-}^{14}\text{C}$]mannose and [$1\text{-}^{14}\text{C}$]glucose in the presence of $^3\text{H}_2\text{O}$

Weights of the mice ranged from 56 to 71 g. Blood glucose concentrations in the fed mice ranged from 10.5 to 11.3 mM and in the fasted mice they were 3.6–6.8 mM. Results are expressed in pmol/h per islet. G, glucose; M, mannose; H, hexose; L, lactate. In column *k*, each value is the average of the ^3H incorporations into glucose in the incubates with [$1\text{-}^{14}\text{C}$]glucose and [$1\text{-}^{14}\text{C}$]mannose multiplied by the fraction of the ^3H bound to C-2 of the glucose as measured by the preparation of an osazone from the glucose isolated in the incubate with [$1\text{-}^{14}\text{C}$]mannose (see the Materials and methods section).

State	Glucose concn. (mM)	$\frac{\text{G} \rightarrow \text{CO}_2}{\text{M} \rightarrow \text{CO}_2}$ (a)	$\frac{\text{G} \rightarrow \text{L}}{\text{M} \rightarrow \text{L}}$ (b)	$\frac{\text{Ratio CO}_2}{\text{Ratio L}} (c = a/b)$	$\frac{\text{G} \rightarrow \text{L} + \text{M} \rightarrow \text{L}}{\text{M} \rightarrow \text{L}}$ (d)	$^3\text{H} \rightarrow \text{L}$ (e)	$\text{M} \rightarrow \text{G}$ (f)	$\text{G} \rightarrow \text{G}$ (g = $a \times f$)	$\text{G} \rightarrow \text{G}$ (h = $b \times f$)	$\text{H} \rightarrow \text{G}$ (i = $f + g$)	$\text{H} \rightarrow \text{G}$ (j = $f + h$)	$^3\text{H} \rightarrow \text{G}$ (k)
Fed ($n = 3$)	5.5	1.8 0.7 0.7	1.1	1.6	20	75	75	135	83	210	158	120
Fed ($n = 2$)	16.7	5.9 2.9	2.6 0.5	2.3 5.8	37 38	95 98	95 129	561 374	247 65	656 503	342 194	177 247
Fasted ($n = 2$)	5.5	0.9 0.8	0.18 0.25	5.0 3.2	124 48	229 189	82 77	74 62	15 19	156 139	95 81	124 176
Fasted ($n = 2$)	16.7	2.1 1.1	1.6 1.1	1.3 1.0	96 44	246 158	118 148	248 163	189 163	366 311	307 311	264 387

not at 16.7 mM (column *b*). The ratios of the yields of $^{14}\text{CO}_2$ /yields of ^{14}C -labelled lactate were, with one exception, greater than 1.0 (column *c*). The incorporation of ^3H from $^3\text{H}_2\text{O}$ into lactate was 2–4-fold greater than the sum of the incorporation of ^{14}C into lactate from $[1-^{14}\text{C}]\text{glucose}$ and $[^{14}\text{C}]\text{mannose}$ (cf. columns *d* and *e*). More lactate was formed in the fasted than in the fed state. The quantity of glucose converted back into glucose via glucose-6-*P* was greater (column *g* compared with column *h*) when considering the CO_2 ratios (eqn. 2) rather than the lactate ratios (eqn. 3) multiplied by the $[1-^{14}\text{C}]\text{mannose}$ converted into glucose (column *f*), except for one experiment in which the ratios were the same. The estimates of the amounts of hexoses converted into glucose (columns *i* and *j*) were correspondingly greater using the CO_2 ratios than using the lactate ratios. Rates of conversion of glucose-6-*P* into glucose estimated from the incorporation of ^3H at C-2 of glucose were in the same range as the rates of conversion of hexose to glucose calculated from the ^{14}C data (Table 2; column *k* compared with columns *i* and *j*).

DISCUSSION

Importance of conversion of mannose into glucose

$[^{14}\text{C}]\text{Mannose}$ is converted into $[^{14}\text{C}]\text{glucose}$ by pancreatic islets from *ob/ob* mice (Tables 1 and 2). Since the only pathway known for the metabolism of mannose in mammalian tissues is through its conversion to fructose-6-*P* [7,18], this is evidence that glucose-6-*P* is dephosphorylated to glucose by islets. In order to conclude that the dephosphorylation occurs in the β -cells of the islets, it must be assumed that the measurements reflect metabolism in those cells. Since over 90% of the cells in the *ob/ob* islets are reported to be β -cells [19], this seems reasonable. The lack of formation of $[^{14}\text{C}]\text{glucose}$ when islets from normal mice were incubated with $[1-^{14}\text{C}]\text{mannose}$ [10,11] may relate to lower glucose-6-phosphatase activity in islets from these mice, or the omission of glucose from the incubation medium. Without unlabelled glucose in the medium to serve as a sink, if labelled glucose had been formed, it could have been utilized. On using $^3\text{H}_2\text{O}$ we observed little glucose cycling in islets from lean mice [5]. Thus we expected, as was the case, to detect no significant increase in ^{14}C incorporation from $[1-^{14}\text{C}]\text{mannose}$ into glucose on incubation of islets from lean mice. This was because of the relatively large amount of ^{14}C -labelled glucose in the incubate in the absence of islets.

Estimates of the extent of glucose-6-*P* hydrolysis

Estimates obtained of the extent of glucose-6-*P* hydrolysis from the incorporation of ^{14}C from $[1-^{14}\text{C}]\text{mannose}$ into glucose and the yields of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]\text{mannose}$ and $[1-^{14}\text{C}]\text{glucose}$ (eqn. 2) are based upon the relationships in Scheme 1. Complete isotopic equilibration of fructose-6-*P* with glucose-6-*P* is assumed, so that the proportion of $[1-^{14}\text{C}]\text{mannose}$ being converted to CO_2 and glucose is the same as for $[1-^{14}\text{C}]\text{glucose}$. Mannose utilization is similarly estimated (eqn. 1), since the ratio of the $^{14}\text{CO}_2$ yield from $[1-^{14}\text{C}]\text{mannose}/^{14}\text{CO}_2$ yield from $[1-^{14}\text{C}]\text{glucose}$ will be proportional to the utilization of these hexoses. While a

Scheme 1. Formation and hydrolysis of glucose-6-*P*

decline in microsomal glucose-6-phosphatase has been observed on incubation of islets [3], the formation of $[^{14}\text{C}]\text{glucose}$ from $[1-^{14}\text{C}]\text{mannose}$ at 90 min was about one-half that at 3 h, supporting the idea that the hydrolysis of glucose-6-*P* proceeds throughout the incubation period.

Estimates of the rates of dephosphorylation of glucose-6-*P* by islets from *ob/ob* mice have been made from the incorporations of ^3H from $^3\text{H}_2\text{O}$ at position C-2 of glucose. It was assumed that in Scheme 2, one atom of ^3H was incorporated at position C-2 of every molecule of glucose-6-*P* formed, because of rapid equilibration of glucose-6-*P* with fructose-6-*P* relative to the rate of conversion of glucose-6-*P* into glucose. Estimated rates of glucose hydrolysis to glucose in the present experiments (hexose $\rightarrow$ glucose in Table 1 and columns *i*, *j* and *k* in Table 2) are 2–3 times greater than the rates estimated using $^3\text{H}_2\text{O}$ in previous studies under similar conditions [4,5]. However, in the present study the amount of glucose-6-*P* hydrolysed that was initially formed from both glucose (at 5.5 or 16.7 mM) and mannose (at 16.7 mM) is estimated, rather than that from glucose alone (at 5.5 and 16.7 mM).

Comparison of estimates made using ^{14}C and $^3\text{H}_2\text{O}$

The advantage with ^{14}C -labelled hexoses is that less radioactivity is required compared with using $^3\text{H}_2\text{O}$. However, estimates using ^{14}C are more time-consuming, with requirements for measuring more than incorporation of label into glucose, and there is the possibility that the presence of mannose alters the metabolism in the islet. However, with regard to the latter possibility, the quantity of hexose utilized (i.e. phosphorylated to hexose-6-*P*) that was subsequently dephosphorylated was about the same (i.e. 32–43%) (Table 1) as the amount of glucose utilized that was dephosphorylated found in previous studies [4,5] (except for a value of 18% in islets from fed mice at 5.5 mM-glucose [5]). The major disadvantage of the use of $[^{14}\text{C}]\text{mannose}$ is the presence of trace amounts of $[^{14}\text{C}]\text{glucose}$, giving backgrounds that make measurements unreliable when there is minimal conversion of mannose into glucose because of the number or source of the islets. In the preparation of $[1-^{14}\text{C}]\text{glucose}$ and $[1-^{14}\text{C}]\text{mannose}$, a mixture is synthesized and then the sugars are separated [20]. Therefore contamination of $[1-^{14}\text{C}]\text{mannose}$ with trace $[1-^{14}\text{C}]\text{glucose}$ was not unexpected. Also, commercial $[1-^{14}\text{C}]\text{glucose}$ contains a 'carbonate-like' impurity [21,22].

Extent of isotopic equilibration of glucose-6-*P* with fructose-6-*P*

The above estimates were made assuming complete isotopic equilibration of glucose-6-*P* with fructose-6-*P*, whether labelled with $[1-^{14}\text{C}]\text{glucose}$ or $[1-^{14}\text{C}]\text{mannose}$. The additional experiments in which lactate was isolated, despite considerable variation from experiment to experiment, provide a measure of this equilibration. As depicted, in Scheme 3, C-1 of glucose-6-*P* is oxidized to CO_2 in the pentose cycle. The other means of $^{14}\text{CO}_2$ formation from $[1-^{14}\text{C}]\text{glucose}$ and $[1-^{14}\text{C}]\text{mannose}$ is in the Krebs cycle. Lactate is formed by glycolysis from fructose-6-*P* and from triose-*P* formed in the pentose cycle. If complete equilibration of glucose-6-*P* with fructose-6-*P* occurs, the proportions of ^{14}C from $[1-^{14}\text{C}]\text{mannose}$ being converted into lactate and CO_2 should be the same as the proportions of ^{14}C from $[1-^{14}\text{C}]\text{glucose}$ being converted into CO_2 and lactate. That is, the ratios in column *c* of Table 2 should have been 1. Five of the six ratios exceeded 1, indicating that there was incomplete isotopic equilibration.

Ratios of incorporations into lactate (column *b*) should be altered very little by the extent of equilibration of the hexose phosphates. This is because the pentose cycle in islets almost certainly makes a relatively small contribution to the utilization

Scheme 2. Glucose cycling via glucose-6-P and equilibration of glucose-6-P with fructose-6-P

Scheme 3. Pentose cycle and formations of CO₂ and lactate

of glucose [13]. Thus most of the ¹⁴C formed from both [1-¹⁴C]glucose and [1-¹⁴C]mannose reaches pyruvate and the glucose → lactate/mannose → lactate ratio is a reflection of the ratio of utilization of glucose and mannose. On the other hand, the ratio of ¹⁴CO₂ yields from glucose and lactate can presumably be markedly altered by the extent of equilibration, since much of the ¹⁴CO₂ formed from [1-¹⁴C]mannose and [1-¹⁴C]glucose by islets is expected to be formed in the pentose cycle [23,24]. If the pentose cycle is active, the specific radioactivity of glucose-6-P will be decreased from what it would be in the absence of that activity. In turn, the extent to which the glucose-6-P specific radioactivity is lessened will determine the extent to which the quantity of glucose-6-P hydrolysed to glucose is underestimated using ¹⁴C.

Effects of non-isotopic equilibration on the estimates

Since the glucose → CO₂/mannose → CO₂ ratios are greater than the glucose → lactate/mannose → lactate ratios, the estimates of glucose → glucose from the former are larger (Table 2, cf. columns *g* and *h*). However, the conversion of [1-¹⁴C]mannose into [1-¹⁴C]glucose is less than it would be if equilibration were complete. Since the lactate ratios are a better reflection of glucose utilization relative to mannose utilization than are the CO₂ ratios, the estimates of mannose utilization in Table 1 obtained from the CO₂ ratios are probably overestimates. As such, total hexose utilization was probably lower than estimated. The estimate of hexose conversion into glucose is then probably between the values calculated using the two ratios (Table 2, columns *i* and *j*).

Effects of fed and fasted states on lactate formation

The amount of hexose utilized was similar in the fed and fasted animals (Table 1). Relatively small decreases in hexose utilization have been observed on fasting [13,25]. The smaller amounts of ¹⁴C and ³H incorporated into lactate in the fed compared with the fasted state (Table 2, columns *d* and *e*) suggest that in the fed state, a smaller percentage of the hexose used was converted into

lactate. This could be related to a presumed decrease in insulin release in the fasted state [25]. However, in other studies, lactate production has been reported to be decreased in the fasted state [13]. The 2–4-fold greater incorporation of ³H compared with ¹⁴C into lactate (Table 2, columns *d* and *e*) means that considerable exchange had occurred between hydrogen atoms in the medium and intermediates in the formation of lactate from the hexoses [26,27].

Effect of glycogen content

The glycogen content of the islets was not measured. If glycogen formation occurred during incubation, the amount of glucose phosphorylated would be underestimated from the yield of ³H₂O from [5-³H]glucose. If there was significant glycogenolysis of glycogen present in the islets before incubation [28,29], the specific radioactivity of glucose-6-P would be diluted when the islets were incubated with the ¹⁴C-labelled hexoses. Hence the estimate of the conversion of glucose-6-P into glucose from ¹⁴C incorporation into glucose would be an underestimate. However, glucose-6-P, whether formed from glucose or glycogen, in the presence of ³H₂O would have ³H bound to its C-2 atom. Hence the incorporation of ³H from ³H₂O into glucose would be a measure of the conversion of glucose-6-P into glucose whether or not there was glycogenolysis. The observation that the estimates with ¹⁴C and ³H are similar indicate that glycogen formation was not affecting the results.

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