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Comparison of xylitol and glucose metabolism in nonhepatic rat tissues

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With 2 tables

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Introduction

Since xylitol ist widely used as an energy source in parenteral nutrition, it is of interest and practical importance to study the ability of different tissues to utilize this polyalcohol.

From the work of *Bässler* et al. (1), *Mehnert* and associates (2) and *Lang* (3) it is well known that xylitol utilized by the human as well as various mammalian organisms. *Müller* et al. (4) reported that in the rabbit about 15 % of the xylitol was utilized by the extrahepatic tissues and 85 % by the liver.

Measuring activities of enzymes involved in xylitol metabolism, *Bässler* et al. (5) and *Hollmann* and *Laumann* (6) demonstrated that liver could be regarded as the main site for xylitol utilization. *Woods* and *Krebs* (7) as well as *Rognstad* and *Katz* (8) carried out extensive studies on xylitol metabolism in isolated, perfused rat liver and hepatocytes. They found that xylitol was rapidly metabolized in the liver, the main products being glucose, lactate and pyruvate. *Wang* and *Meng* (9) studied the extent of xylitol metabolism in liver and various extrahepatic minced tissues of the rat in vitro, using ^{14}C-labelled substrate and following the incorporation of radioactivity into CO_2 and lipids. From their results they concluded that the extrahepatic tissues of rats, excluding muscle, were capable of metabolizing xylitol in the in vitro test system. Since these authors obtained only small amounts of radioactivity incorporated into the endproducts in all tissues except liver, kidney and lung and because they did not measure directly the uptake of xylitol, we decided to reinvestigate the problem of xylitol utilization using two different analytical methods. In addition we compared in all experiments the extent of xylitol utilization directly with that of glucose. The present study was carried out in order to find out which tissues, except liver, contribute significantly and directly to the metabolism of xylitol. This information is important in view of the existence of minor pathways leading to toxic compounds like oxalic acid as has been reported by *Thomas* et al. (10).

We report here our results of xylitol and comparatively glucose metabolism in nonhepatic tissue slices, obtained by isotopic and enzymatic analyses.

Materials and Methods

Chemicals: Enzymes, coenzymes, nucleotides and biochemical substrates used were obtained from Boehringer, Mannheim, FRG. [U-^{14}C]-xylitol and [U-^{14}C]-glucose were purchased from Amersham Buchler, Braunschweig, FRG. All other chemicals were of reagent grade and products from Merck, Darmstadt, FRG.

Krebs-Ringer phosphate buffer (11) pH 7.4 free of calcium ions was used for incubation of slices. The *Krebs-Henseleit* bicarbonate buffer (12) pH 7.4 taken in case of fat cells contained 2% bovine serum albumin. Both buffers were gassed during handling with $O_2 + CO_2$ (95 + 5).

Animals: All tissues used were obtained from normal fed male rats of a Sprague Dawley strain, 180–220 g in weight.

The animals were killed by decapitation and the organs rapidly prepared, washed and sliced. The following organs were used in separate experiments: brain, lung, heart, kidney and skeletal muscle. Fat cells were prepared as described by *Rodbell, M.* (13) except that glucose was omitted from the medium during the collagenase treatment and washing procedures. Plastic equipment was used throughout the cell preparation.

Incubation: For investigation of slices about 300 mg of sliced tissue (500 mg in case of lung) was incubated at 37° C in stoppered 100 ml Erlenmeyer flasks. 2 ml of fat cells suspended to a final concentration of 80 µmol of triglyceride content/ml were incubated in stoppered plastic scintillation vials. All incubations were performed under $O_2 + CO_2$ (95 + 5) and all flasks contained a vial for CO_2 collection. The incubation was started by adding the substrate glucose (5 µmol, in case of brain 10 µmol and 1 or 2 µCi) or xylitol (5 µmol and 1 or 2 µCi). The incubation was stopped after 60 min (in case of fat cells after 120 min) by injection of perchloric acid. $^{14}CO_2$ was trapped by 2-phenylethylamine. Shaking was continued for 45 min for quantitative $^{14}CO_2$ collection.

Analytical procedures: In the neutralized perchloric acid extracts (20–22 ml) glucose, xylitol and lactate were determined enzymatically. Xylitol was assayed with sorbitol dehydrogenase (EC 1.1.1.14) in the following assay: 0.2 ml of 120 mM NAD, 1 ml of glycine semicarbazid buffer (1 M + 0.2 M), pH 9.0 and up to 0.2 ml of sample, to give a change in optical density in the range of 0.15 to 0.3.

The assay was started by the addition of 0.01 ml of a sorbitol dehydrogenase suspension (5 ml/ml).

For isotopic determination of metabolites and substrates the method used was as described by *Katz* et al. (14). 2 ml of the neutralized perchloric acid extract were passed through a column (1 × 10 cm) of Lewatit M 5080 (Merck, Darmstadt, 100–200 mesh) in the acetate form. The columns were eluted with 6 × 10 ml of water. This fraction consists of neutral compounds. The elution was proceeded by 6 × 10 ml of 1 N formic acid to elute lactate, 6 × 10 ml of 4 N formic acid to elute pyruvate and 6 × 10 ml of mixture containing 2 N formic acid and 4 N ammonium formiate. This removes the highly negatively charged compounds such as phosphate esters and di- and tricarboxylic acids. By these elutions all radioactivity (96–102%) applied to the column could be recovered in the various fractions. From the radioactivity found in the pooled fractions the amount of substrate uptake and the formation of metabolites and products could be calculated.

To calculate glucose formation from xylitol in experiments with kidney slices 2 ml of the neutralized perchloric acid extract were treated with excess ATP and hexokinase (EC 2.7.11) in order to convert glucose completely to glucose-6-phosphate. This compound is not eluted with water and therefore the collected radioactivity in the neutral fraction corresponds to the not utilized ^{14}C-xylitol.

The difference in radioactivity in the neutral fraction before and after treatment with hexokinase and ATP is used in calculating the extent of gluconeogenesis from xylitol.

Lipids were extracted from the insoluble residues with chloroform-methanol (2 : 1, v/v) and the crude extracts were purified as described by *Folch* et al. (15).

Results

Xylitol and glucose utilization by various extrahepatic rat tissues

Table 1 shows the extent of xylitol utilization by various extrahepatic rat tissues in comparison to that of glucose and the formation of the main products during the incubation period. Two different methods involving isotopic techniques and enzymatic analyses have been applied in order to determine the levels of the substrates and products. From the isotopic data only the amount of ^{14}C-labelled substrates taken up and that of ^{14}C-labelled products formed can directly be calculated. Measurements of substrate and product concentrations by enzymatic analyses yield the actual concentrations without discriminating their origin from added ^{14}C-labelled substrates or endogenous sources.

While the isotopic method allows only to determine the degradation rates of added ^{14}C-labelled substrates, the comparison of both methods gives additional information about the activity of other metabolic pathways, such as gluconeogenesis and glycogenolysis.

Kidney

As can be seen from table 1, kidney is the only extrahepatic organ which is able to utilize xylitol significantly. In all other extrahepatic tissues tested there was no detectable uptake of xylitol using both methods. From the amount of ^{14}C-glucose taken up, 84 % could be recovered in the products ^{14}C-lactate, ^{14}C-pyruvate and $^{14}CO_2$. Glucose uptake determined by isotopic measurement was found to be 19.6 μg atom C/g/60 min, while by enzymatic analyses a net uptake of only 9.4 μg atom C/g/60 min has been obtained. The difference of 10.2 μg atom C of glucose indicates the formation of glucose during the incubation period by glycogenolysis and/or gluconeogenesis, most likely from lactate, as the concentration of which was found to be decreased. ^{14}C-xylitol uptake amounted one third that of ^{14}C-glucose and part of it was found to be converted to glucose.

Lung

Glucose uptake by the lung was slightly higher than that of the kidney, staying in the same range with both methods, thus excluding formation of glucose from endogenous sources. Lactate formation determined by enzymatic analyses exceeded that determined by isotopic method almost by a factor of two, indicating lactate formation from endogenous compounds. This is supported by the fact that lactate was also produced with xylitol as substrate. Xylitol uptake was below the validity of the methods which is in accordance to the very small amounts of radioactivity which were found to be incorporated into the products lactate, lipids and CO_2.

Brain

A very active glucose metabolism could be observed with brain slices while again xylitol was not utilized by this tissue. The fact that 30 % of

Table 1. Comparison of xylitol and glucose metabolism in nonhepatic rat tissues.

Mean values of 3–4 experiments obtained from isotopic and analytical methods are expressed as μgatom carbon · g^{-1} tissue (ww) · 60 min^{-1} with the ranges in parenthesis. Substrate uptake smaller than 5% of the amount of substrate added is within the error of the methods and therefore not detectable. In case of isolated adipocytes the values are referred to 100 μmol triglycerides. For incubation procedures, experimental details and calculations see Material and methods section.

Tissue	Substrate	Isotopic (μgatom C/g/60 min) ^{14}C-Glucose	% recovered	^{14}C-Xylitol	% recovered	Analytical (μgatom C/g/60 min) Glucose	Xylitol
Kidney	Uptake	19.6 (16.74–22.37)		6.6 (6.55–6.65)		9.4 (5.12–12.41)	5.5 (0.37–8.72)
	Δ Lactate	+4.7 (4.01–5.1)	24	+0.7 (0.61–0.77)	11	−5.3 (−5.24to −5.55)	−6.8 (−5.69to −7.61)
	Δ ^{14}C-Pyruvate	+1.14 (0.93–1.22)	6	not detectable	–		
	$^{14}CO_2$ production	10.6 (8.97–11.79)	54	1.8 (1.43–2.01)	28		
	^{14}C-Glucose formation			2.3 (1.97–2.75)	35		
Lung	Uptake	23.2 (22.13–25.31)		not detectable		26.9 (22.18–29.28)	not detectable
	Δ Lactate	+14.8 (14.04–15.98)	64	+0.51 (0.49–0.57)	–	+26.1 (24.61–28.00)	+14.1 (13.71–14.59)
	Δ ^{14}C-Pyruvate	+0.7 (0.56–0.77)	3	not detectable	–		
	Lipid formation	+0.7 (0.64–0.84)	3	+0.05 (0.047–0.062)	–		
	$^{14}CO_2$ production	3.5 (3.0–3.89)	15	+0.15 (0.14–0.16)	–		
Brain (cortex)	Uptake	114.0 (105.04–129.28)		not detectable		134.4 (124.61–154.36)	not detectable
	Δ Lactate	+72.0 (64.09–85.54)	63	+0.4 (0.29–0.98)	–	+33.8 (29.61–44.78)	−17.4 (−15.67 to −20.57)
	$^{14}CO_2$ production	34.7 (32.67–37.43)	30	0.09 (0.07–0.1)	–		

Tissue	Substrate	Isotopic (μgatom C/g/60 min) ^{14}C-Glucose	% recovered	^{14}C-Xylitol	% recovered	Analytical (μgatom C/g/60 min) Glucose	Xylitol
Heart muscle	Uptake	16.6 (13.04–21.26)		not detectable		14.8 (7.84–17.39)	not detectable
	Δ Lactate	+8.5 (6.78–10.29)	51	+0.3 (0.2–0.36)	–	+24.3 (11.15–30.95)	+18.8 (9.09–26.4)
	Δ ^{14}C-Pyruvate	+3.72 (2.52–5.03)	22	not detectable	–		
	$^{14}CO_2$ production	1.13 (0.81–1.65)	7	0.02 (0.017–0.022)	–		
Skeletal muscle	Uptake	10.3 (9.07–12.34)		not detectable		not detectable	not detectable
	Δ Lactate	8.2 (7.21–9.38)	80	0.15 (0.11–0.21)	–	+122.3 (118.25–127.71)	110.5 (95.56–121.04)
	$^{14}CO_2$ production	1.49 (0.95–1.94)	15	0.012 (0.010–0.014)	–		
		μgatom C/100 μmol triglyceride/60 min				μgatom C/100 μmol triglyceride/60 min	
Adipocytes	Uptake	3.9 (3.6–4.14)		not detectable		5.1 (4.81–5.46)	not detectable
	Δ Lactate	+1.3 (0.96–1.59)	35	not detectable	–	+1.3 (0.90–1.75)	−0.08 (−0.07 to −0.09)
	$^{14}CO_2$ production	1.12 (0.92–1.34)	29	0.01	–		
	Lipid formation	1.56 (1.38–1.77)	40	0.02 (0.018–0.024)	–		

the glucose carbon atoms could be recovered in CO_2 indicates an active oxidative metabolism. This is confirmed also by the finding that the lactate level decreased in the presence of xylitol as substrate which was not taken up. It should be noted that there is a discrepancy between the isotopic and the analytical data in the case of lactate. 72 µg atom C have been found isotopically in the lactate fraction while analytically the lactate concentration increased only by 33.8 µg atom C. In order to elucidate this difference the concentration of lactate in the isotopic lactate fraction was determined enzymatically and found to be 47 µg atom C/g/60 min. Therefore, the difference of 25 µg atom must be attributed to a compound with similar acidity which coelutes from the anion exchange column with lactate.

Muscle

Table 1 shows that 80 % of the ^{14}C-glucose carbon atoms taken up by heart muscle were metabolized to ^{14}C-lactate, ^{14}C-pyruvate and $^{14}CO_2$ while xylitol was not utilized to a measurable extent. In the case of skeletal muscle almost all ^{14}C-glucose taken up could be recovered in ^{14}C-lactate and $^{14}CO_2$. The analytical data revealed an extensive formation of lactate in both types of muscle when glucose or xylitol was added as substrate. The excess lactate formed must be derived from endogenous sources, most likely from glycogen. The fact that enzymatic analyses could detect no glucose uptake in slices of skeletal muscle cannot be explained, since it is generally accepted that glucose formation via glycogenolysis does not occur in muscle tissue.

Adipocytes

Adipocytes obviously are not capable of utilizing xylitol since no measurable uptake could be detected and only neglectable amounts of radioactivity were found to be incorporated in endproducts such as lipids and CO_2. Glucose, on the other hand, using both methods, proved to be a suitable substrate being converted to lipids, lactate and CO_2.

Table 2. Recovery of radioactivity in CO_2 and lactate from ^{14}C-xylitol as compared to ^{14}C-glucose (= 100%)

For the calculation the isotopic data conferred in table 1 are taken. For comparison of the values obtained with ^{14}C-glucose and ^{14}C-xylitol the incorporation of radioactivity into CO_2 and lactate from ^{14}C-glucose is set 100%.

Rat tissue	$^{14}CO_2$ (% of glucose)	^{14}C-lactate (% of glucose)
Kidney	17	15
Lung	4.3	3.4
Brain (cortex)	< 1	< 1
Heart muscle	1.7	3.5
Skeletal muscle	< 1	1.8
Adipocytes	< 1	< 1

Comparison of xylitol and glucose metabolism

Table 2 shows the extent of xylitol metabolism of various extrahepatic tissues in comparison to that of glucose, as judged from the incorporation of radioactivity into CO_2 and lactate. The values obtained with ^{14}C-glucose are set 100 %. The data clearly show that of all nonhepatic tissues tested, only kidney is able to utilize xylitol to a considerable extent. All other tissues produced less than 5 % of $^{14}CO_2$ and ^{14}C-lactate from ^{14}C-xylitol as compared to glucose and therefore they do not contribute to xylitol metabolism in the whole organism.

Discussion

The purpose of this work was to study which organs of the rat are capable of metabolizing xylitol directly and to what extent. Since *Wang* and *Meng* (9), who carried out similar studies, have found only very small amounts of radioactivity incorporated from ^{14}C-xylitol into products like CO_2 and lipids in most of the tissues studied, we found it necessary to reinvestigate this problem using comparable methods but in addition also to measure directly the uptake of xylitol. Our results clearly show that from all nonhepatic tissues tested only kidney is able to utilize xylitol to a significant extent. Even when applying two different methods, no uptake of xylitol could be measured in all the other tissues. Since these methods do not allow the detection of less than 5 % uptake of added substrate, we also followed the incorporation of radioactivity from ^{14}C-labelled substrate into products like CO_2 and lactate. This is an extremely sensitive method to study metabolic degradation of a given substrate. The results of these measurements also indicate that xylitol utilization in all tissues studied, except kidney, is neglectable. This interpretation of our results is not consistent with the statement of *Wang* and *Meng* (9) who concluded from their results that extrahepatic tissues of rats, except muscle tissue, were capable of metabolizing xylitol in the in vitro system using CO_2-production and lipid synthesis as criteria. The numeric values of $^{14}CO_2$-production from ^{14}C-xylitol (nmol/g/60 min) obtained in our experiments are comparable with those reported by *Wang* and *Meng* (9). For convenience a comparison of relative values calculated from the results of *Wang* and *Meng* and of our results is presented.

The percentage values for the incorporation of ^{14}C-xylitol into the products CO_2 and lipids from added substrate reported by these authors are 0.42 %*) and 0.25 %*) respectively in case of lung and 0.07 %*) and 0.24 %*) in case of the fat pads.

The corresponding percentage values calculated from our results are 0.3 % and 0.1 % in case of lung and 0.03 % and 0.13 % in case of adipocytes. From these values it can be seen that in both laboratories extremely low incorporation rates (far below 1 % of added substrate) of ^{14}C-xylitol into these products have been obtained and therefore by no means a capability

*) The percentage values given by *Wang* and *Meng* in tables 1 and 3 of their paper (8) have to be devided by 3.3 in order to correct for 0.3 g of tissue used in their incubation system. It should be noticed that the values given in their paper are the percentage values of initial amount of xylitol added but not utilized since xylitol uptake has not been determined.

of these tissues for xylitol metabolism can be deduced. This conclusion is further supported by the fact that no xylitol uptake was detectable with the exception of kidney tissue. In addition when the production of CO_2 and lactate from ^{14}C-xylitol in the various extrahepatic tissues is compared to that of ^{14}C-glucose (table 2) it is obvious that only kidney is able to metabolize xylitol significantly and that the values obtained in the other tissues might be due to contamination of the tissue with other cells e.g. red blood cells which have been shown to be able to utilize xylitol (16, 17).

Petrich et al. (18) reported that all enzymes of the glucuronic acid-xylulose-cycle are present in adipose tissue in relatively high activities and therefore concluded that xylitol could be utilized by adipose tissue in substantial amounts. It is, however, not correct without further metabolic studies to postulate from measurements of enzyme activities the existence of an active xylitol metabolism. Since our results exclude a significant contribution of the adipose tissue to xylitol metabolism, it seems to be unlikely that the antilipolytic effect of xylitol observed by *Opitz* (19) is due to an enhanced formation of glycerol-1-phosphate in adipose tissue in the presence of xylitol, but rather due to an enhanced lipoprotein synthesis induced by xylitol in the liver. Recently *Mader* and *Reinauer* (20) studied xylitol utilization in isolated perfused heart of rat and reported that only 3–4 % of the oxygen-consumption could be referred to xylitol utilization.

They further state that with xylitol as the only substrate, the heart is working under substrate deficiency conditions and therefore xylitol is not a suitable substrate for the heart muscle, a conclusion which also can be drawn from our in vitro studies.

Opitz and *Lutz* (21) have shown that xylitol can be found in the cerebral liquor during xylitol infusion but they state correctly that this does not necessarily mean that xylitol can be utilized by the brain. In order to follow this problem they studied xylitol metabolism in brain homogenates. They found that xylitol uptake in brains obtained from hypoglycaemic rats is enhanced compared to that from normoglycaemic rats (135 and 91 μg/g/h respectively).

Calculating their results on the basis of our experimental conditions (0.3 g tissue and incubation time of 60 min) xylitol uptake amounts to 1 μg atom C/g/h. We, however, were not able to detect xylitol uptake in brain tissue by isotopic and enzymatic analyses. From the formation of the products lactate and CO_2, determined by isotopic measurements, one could deduce an uptake of xylitol in the range of 0.5 μg atom/g/h which is close to the results reported by *Opitz* and *Lutz*. These values, however, are extremely low and clearly indicate that also for brain, xylitol is not a suitable substrate. This interpretation is further justified by our finding that product formation from xylitol as substrate in the brain is less than 1 % of that from glucose. Recently *Stein* and *Cohen* (22) investigated CO_2-production in rat brain slices from glucose, fructose and xylitol as substrate using microtitration techniques. They found that xylitol was not metabolized to CO_2, a result which is completely consistent with our measurements.

With mouse pancreatic islets *Ashcroft* et al. (23) reported that [U-^{14}C]-xylitol gave rise to only small amounts of $^{14}CO_2$ which, compared to that from [U-^{14}C]-glucose, can be neglected. It should be emphasized that it is extremely difficult to rule out the possibility of any xylitol utilization in the tissues because of the limitations of the methods available. In almost all tissues studied we as well as other investigators could find small amounts of ^{14}C-labelled products which must have been derived from ^{14}C-xylitol. Since these rates are extremely low, it is in our opinion not justified to interpret these data in terms of a xylitol metabolizing capability of the tissues. The main point of our studies was to find out for which organ xylitol is a suitable substrate, contributing significantly to the energy metabolism.

From this point of view our results as well as the results of all other authors referred to clearly indicate that with the exception of liver only kidney can utilize xylitol to a significant extent.

Summary

Capability and extent of xylitol metabolism in vitro was studied in a variety of non-hepatic tissues of rats, using enzymatic and isotopic methods. Only kidney was found to be able to utilize xylitol at a significant rate while in all other organs tested – lung, brain, heart muscle, skeletal muscle and adipose tissue – no uptake of xylitol was detectable using two independent methods.

In accordance with these results the incorporation of radioactivity from [U-^{14}C]-xylitol as substrate into the products CO_2 and lactate was found to be very small in all organs with the exception of kidney and when compared to that from [U-^{14}C]-glucose it was less than 5 %. From these results it is concluded that besides liver only kidney can utilize xylitol to a significant extent while for the other tissues studied xylitol is not a suitable substrate.

Zusammenfassung

Es wurde untersucht, ob und in welchem Ausmaß eine Reihe isolierter, nicht-hepatischer Rattengewebe Xylit verwerten. Nur die Nierenschnitte waren in der Lage, Xylit signifikant zu verwerten, während bei Schnitten von Lunge, Hirn, Herz- und Skelettmuskulatur sowie bei Fettzellen weder mit enzymatischer Analytik noch mit Isotopentechniken eine Xylitaufnahme direkt nachweisbar war.

Übereinstimmend mit diesem Ergebnis war der Einbau von Radioaktivität aus [U-^{14}C]-Xylit in CO_2 und Lactat außer bei den Nierenschnitten sehr gering und verglichen zum Einbau aus Glucose (= 100 %) weniger als 5 %. Aus den Ergebnissen wird geschlossen, daß, abgesehen von der Leber, nur die Niere in der Lage ist, Xylit zu verwerten, während er für die anderen untersuchten Organe keine geeignetes Substrat darstellt.

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