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Effects of Glucose on the Metabolization of Fructose and Glycerol by Isolated Adipocytes from Rat *

J. Bellido and E. Herrera **

Departamento de Fisiología, Facultad de Medicina,
Universidad de Sevilla and Servicio de Bioquímica,
Departamento de Investigación,
Centro Especial Ramón y Cajal
Madrid-34 (Spain)

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Isolated white fat cells were incubated in medium containing (^{14}C)-fructose (UL), supplemented with either 1 or 5 mM fructose, in the presence or absence of 5 mM glucose. The utilization of fructose for the formation of CO_2, fatty acids or glyceride glycerol was very much dependent on its concentration in the medium. It was significantly inhibited by glucose only at fructose concentration of 5 mM. When the cells were incubated in the presence of (1-^{14}C) glycerol, fructose produced a significant increase in the conversion of the tracer to CO_2, fatty acids and glyceride glycerol, while glucose produced an increase in the formation of both CO_2 and fatty acids, but a significant decrease of glyceride glycerol from the same labelled precursor. Glucose causes the effects of fructose, enhancing the uptake of labelled glycerol by the cells, to disappear.

Fructose is metabolized by adipose tissue by means of its phosphorylation to fructose-6-phosphate (15). The administration of large amounts of fructose leads to an increase in the concentration of triglycerides in blood (17-19, 22), and this effect has been related with an enhanced release of free fatty acids from adipose tissue as a consequence of reduced fatty acid re-esterification (23). Glucose seems to be the main source of α-glycerophosphate for the fatty acid esterification in adipose tissue, but it has been shown that glyceride glycerol can also be formed in this tissue from other substrates, such as pyruvate, certain amino

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** To whom all correspondence should be addressed: Departamento de Investigación, Centro Ramón y Cajal, Carretera de Colmenar, Km 9. Madrid - 34 (Spain).

acids, fructose and glycerol (1, 3-5, 10-12, 24). Thus, fructose can affect the rate of fatty acid esterification in adipose tissue, either by acting as a substrate for the α-glycerophosphate synthesis, or by altering the glycerol reutilization rate by the tissue. In the present study we have analyzed the metabolism of fructose by isolated fat cells *in vitro*, how this is affected by glucose, and the comparative effects of both fructose and glucose on the utilization of glycerol by the same preparation.

Materials and Methods

White fat cells were obtained from the parametrial adipose tissue of 158-189 g female rats fed *ad libitum* on laboratory rat chow. The adipocytes were isolated (20) following the methodology described in the previous paper (1), by using defatted (2) bovine serum albumin, glucose, ovomucoid trypsin inhibitor and collagenase. The cells were quantified according to the protein concentration (14, 16) of their suspensions. They were incubated for 120 min, as previously described (1), in Krebs Ringer bicarbonate buffer containing defatted bovine serum albumin (20 mg/ml) and 0.5 μCi of either (^{14}C)-fructose (UL) (210 mCi/mmol), or (1-^{14}C) glycerol (31 mCi/mmol)/ml, supplemented or not with non-radioactive fructose or glucose. Glycerol was determined (9) in the media and the lipids were separated, extracted (6) and fractionated (4), as previously indicated.

Results

Metabolization of fructose by isolated fat cells. Fructose utilization by isolated fat cells incubated *in vitro* was very much dependent on its concentration (table I). Actually, the amount of fructose oxidized to CO_2, or converted to either fatty acids or glyceride glycerol, increased with the increase in the amount of substrate in the medium, the smallest change being observed in the formation of glyceride glycerol. 5 mM glucose decreases very slightly the amount of fructose metabolized by the adipocytes when the concentration of this hexose in the medium was 1 mM, but the effect was much greater when the fructose concentration in the medium was 5 mM. The presence of glucose in the medium, however, did not affect the

Table I. *Effect of glucose on the utilization of (^{14}C)-fructose (UL) by isolated white fat cells from fed rats.*

Values correspond to means ± S.E.M., of 8 rats/group. *p* values correspond to the statistical differences between the samples incubated in the presence and those in the absence of glucose, while *p'* values correspond to the differences between the samples incubated in the presence of 5 mM fructose and those in that of 1 mM fructose.

	nmoles of fructose utilized/100 μg of cell proteins					
Additions to the medium	CO_2	P	Fatty acids	P	Glyceride glycerol	P
Fructose (1 mM)	11.9±0.3		7.50±0.72		6.81±1.25	
Fructose (1 mM) + Glucose (5 mM)	10.5±0.4	< 0.05	9.18±0.32	N.S.	4.58±0.53	N.S.
Fructose (5 mM)	56.7±0.4		54.5±2.52		23.8±4.0	
P'	< 0.001		< 0.001		< 0.01	
Fructose (5 mM) + Glucose (5 mM)	32.0±2.1	< 0.001	32.2±2.9	< 0.001	12.5±1.6	< 0.05
P'	< 0.001	< 0.001	< 0.001		< 0.001	

Table II. *Effect of fructose and glucose on the utilization of (1-^{14}C)-glycerol by isolated white fat cells from fed rats.*

Values correspond to means ± S.E.M., of 5/9 rats/group. *p* values correspond to the statistical differences between each group and that of the samples incubated with «no additions» to the medium.

Additions to the medium	% Initial radioactivity in the incubating medium/100 μg of cell proteins							
	(^{14}C)-glycerol uptake	p	$^{14}CO_2$	p	^{14}C-glyceride glycerol	p	^{14}C-fatty acids	p
None	1.29±0.29		0.35±0.03		0.07±0.01		0.87±0.04	
Fructose (5 mM)	2.32±0.31	<.05	0.81±0.04	<.001	0.35±0.03	<.001	1.15±0.06	<.001
Glucose (5 mM)	1.51±0.19	N.S.	0.52±0.03	<.001	0.26±0.01	<.001	0.70±0.03	<.01
Glucose (1 mM) + Fructose (4 mM)	1.21±0.17	N.S.	0.36±0.01	N.S.	0.22±0.02	<.001	0.54±0.03	<.001
Glucose (2 mM) + Fructose (3 mM)	0.99±0.25	N.S.	0.33±0.01	N.S.	0.21±0.01	<.001	0.45±0.02	<.001
Glucose (3 mM) + Fructose (2 mM)	0.99±0.16	N.S.	0.36±0.01	N.S.	0.20±0.01	<.001	0.44±0.02	<.001
Glucose (4 mM) + Fructose (1 mM)	1.08±0.19	N.S.	0.36±0.02	N.S.	0.21±0.01	<.001	0.50±0.03	<.001
Glucose (5 mM) + Fructose (5 mM)	1.25±0.37	N.S.	0.49±0.04	<.05	0.29±0.02	<.001	0.47±0.03	<.001

proportional conversion of glucose to CO_2, fatty acids and glyceride glycerol.

Comparative effects of glucose and fructose on glycerol metabolization by isolated fat cells. In table II are shown the values of (^{14}C) glycerol utilization by isolated fat cells incubated in the presence of either 5 mM fructose, 5 mM glucose or the combination of both hexoses at different concentrations to give a final concentration of 5 mM. Fructose alone produces a significant increase in the uptake of labelled glycerol by the cells, this effect being accounted by the conversion of glycerol to CO_2, fatty acids and glyceride glycerol. Different from fructose, glucose alone in the medium does not produce a change in the uptake of labelled glycerol by the cells, although it produces a significant increase in the conversion of glycerol to both CO_2 and fatty acids and a significant decrease in its conversion to glyceride glycerol. The presence of different amounts of both glucose and fructose in the medium to give a final concentration of 5 mM or the presence of 5 mM concentration of each of the two hexose prevents the effects of fructose alone enhancing the uptake of (^{14}C) glycerol by the cells. They also produce a diminution of the conversion of glycerol to either CO_2 or glyceride glycerol as compared with the values observed with either hexose alone, and no change in their effect on fatty acid synthesis from the same substrate.

The presence of fructose in the incubation medium did not affect the amount of glycerol produced by the adipocytes during the incubation at any of the concentrations studied.

Discussion

In agreement with other authors (6-8, 15), the present results indicate that most of the fructose metabolized by adipose tissue *in vitro* is used for fatty acid synthesis and its oxidation to CO_2, a small portion being used for glyceride glycerol synthesis. These relationships differ from those observed for glucose (3, 7, 8) in the sense that with this hexose a higher proportional conversion to glyceride glycerol than from fructose is observed. Fructose is directly phosphorylated to fructose-6-phosphate (15) and glucose to glucose-6-phosphate (13). Probably the comparative utilization by the glycolytic, the hexose monophosphate and the glycogenetic pathways differ between both hexoses, which would explain their different conversion to glyceride glycerol.

It is observed that glucose inhibits the metabolization of fructose by isolated adipocytes, which does not agree with the results of FROESCH and GINSBERG (7) in epididymal fat pad pieces. Besides the different preparations used, the difference could be a consequence of the change of sensitivity to the effect of glucose depending on the relative concentration of both fructose and glucose. Actually, the inhibitory effect of glucose was not observed here at 1 mM fructose concentration in the medium. It has been suggested that glucose and fructose are transported into the adipose tissue cell in an independent manner (7), but the fact that 5 mM glucose inhibits the metabolization of 5 mM fructose indicates that some competitive relationship must exist in the transport and/or metabolization of both hexoses. According to the described K_m value of hexokinase for the phosphorylation of either hexose in adipose tissue (13), our results would suggest that glucose influences the utilization of fructose just when the concentration of the latter is above the K_m value of hexokinase for it (13). This mutual competence is also suggested by the results on their effects on glycerol metabolism, that are not additive when both hexoses are present in the incubation medium.

The effects of glucose enhancing the formation of CO_2 and fatty acids from glycerol and decreasing its conversion to glyceride glycerol without changing the net uptake of this substrate, confirm the results previously observed in pieces of epididymal fat pads (4). Different from glucose, fructose enhances the uptake of labelled glycerol as it increases its conversion to all the studied parameter, including the glyceride glycerol. The metabolization of glycerol by adipose tissue *in vitro* depends on the availability of ATP for its phosphorylation (5), which could be apported by either fructose or glucose. The low proportional conversion of fructose to glyceride glycerol allow to suggest that contrary to glucose, the amount of α-glycerophosphate formed from fructose is not enough to compete with that formed from glycerol for the esterification of fatty acids. This hypothesis agrees with the effect of fructose enhancing the inhibitory effect of glucose on the synthesis of glyceride glycerol from glycerol. In this condition, the additive metabolism of both hexoses in adipose tissue (7) would produce a maximal amount of α-glycerophosphate and a greater competence with that formed from glycerol.

So far, the present results can not be extrapolated to the *in vivo* situation, but the direct effect of fructose on glycerol adipose metabolism described herein must be taken into account to understand the overall effect of fructose feeding enhancing the plasma concentration of triglycerides (17-19, 23). Actually, the augmented glycerol metabolization by adipocytes incubated in the presence of fructose justifies the reduced plasma free fatty acids and glycerol levels observed by other authors (21) shortly after fructose load. However, it does not explain the reduced

re-esterification of fatty acids suggested by other authors (22) in animals fed with high fructose diet for a prolonged period. There exists the possibility that the lipogenetic effect of this hexose and its own use as lipogenetic substrate may be responsible for the augmented release of free fatty acids from the adipose tissue of animals fed with fructose (22).

Resumen

Se incubaron adipocitos aislados de rata en medio conteniendo fructosa-U-C^{14}, suplementado con fructosa 1 ó 5 mM, en presencia o en ausencia de glucosa 5 mM. La formación de CO_2, ácidos grasos o glicerol de glicéridos a partir de fructosa era dependiente de la concentración de substrato, siendo inhibida por la glucosa solamente a concentraciones 5 mM de fructosa. Cuando las células se incubaron en presencia de glicerol-1-C^{14}, la fructosa produjo un aumento significativo de la conversión del trazador a CO_2, ácidos grasos y glicerol de glicéridos. Sin embargo, la glucosa produjo un aumento de la formación de CO_2 y ácidos grasos, pero un descenso significativo de la formación de glicerol de glicéridos, a partir del mismo precursor radioactivo. La glucosa produjo la desaparición del efecto de la fructosa aumentando la captación de glicerol radioactivo por las células.

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