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The adenine nucleotide content of rat liver during infusions of carbohydrates and polyols

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With 2 tables

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Intensive studies of the metabolism and metabolic effects of various carbohydrates and polyols with respect to their use in parenteral nutrition have led to the observation of "side effects" such as increases of serum bilirubin or uric acid (1, 2).

In seeking the cause of the uric acid increase attention was focussed on liver adenine nucleotides as a possible source. A group of Finnish authors (3, 4) showed a loss of liver ATP and total adenine nucleotides as well as of inorganic phosphate after rapid injection of some carbohydrates, especially fructose. *Woods* et al. (5) observed the same phenomenon during perfusion of the isolated liver with fructose. It was concluded that the drop of ATP and inorganic phosphate increased the breakdown of adenine nucleotides to uric acid, since ATP inhibits 5-nucleotidase and P_i inhibits AMP deaminase (5). IMP, a degradation product of adenine nucleotides, inhibits the fission of fructose 1-phosphate by liver aldolase (5). The changes in liver metabolism described in this series of papers caused reluctance to use fructose in infusion therapy and even in the diet (6) in spite of the fact that fructose for many years has been successfully used even in the treatment of liver diseases (7).

This situation induced us to take up these studies again and to repeat them under more physiological conditions. Injection of a large dose of fructose is not a physiological event. It can be anticipated that the rapid phosphorylation following fructose injection would disturb the homeostasis of liver adenine nucleotides, but this might soon be reestablished during continuous infusion. This proved to be true when we compared the behaviour of liver adenine nucleotides after rapid injection of carbohydrates with the situation during continuous infusion.

Materials and methods

Male SIV-rats weighing 170–240 g obtained from *S. Ivanovas* (7967 Kisslegg, West-Germany) were used. They were fed on a standard pellet diet (Altromin) ad libitum until 18 hrs before the beginning of the experiment.

A small catheter was inserted in the inferior vena cava exposed at laparotomy under ether anaesthesia. The abdomen was closed carefully and the experiment did not begin until 1 hour later when the animal had completely recovered from anaesthesia.

In one set of experiments 1 mmole of carbohydrate in 2 ml was injected through the catheter in 20 seconds. Controls received 2 ml Tyrode's solution.

In another series of experiments carbohydrates were infused continuously by means of an infusion pump (UNITA I, Braun-Melsungen). The rate of infusion of carbohydrate was 1,5 $g \cdot kg^{-1} \cdot h^{-1}$ in a volume of 1,2 ml/h. Controls received an equivalent volume of Tyrode's solution.

If carbohydrate mixtures were used, equal parts of the components were added so that the total amount was 1 mmole in injections and 1,5 $g \cdot kg^{-1} \cdot h^{-1}$ in infusions.

5 min after the injection or 30, 60, 120 and 180 min after the start of the infusion liver samples were removed by the freeze-stop method (8) and analyzed for ATP (9), ADP, AMP (10) and inorganic phosphate (11, Merckotest-kit).

Reagents and enzymes for the determination of adenine nucleotides were purchased from Boehringer-Mannheim. Xylitol was a gift from J. Pfrimmer and Co., Erlangen. All other substances and reagents were obtained from E. Merck, Darmstadt.

Results are expressed as means with the standard deviation in brackets. The statistical significance of differences between means was examined by Student's t-test at the 1% level.

Results

Table 1 summarizes the contents of adenine nucleotides and P_i in rat livers 5 min after the injection of 1 mmole of various carbohydrates compared with controls which received Tyrode's solution.

The results are in agreement with those of *Raivio* et al. (4). There is a pronounced and highly significant fall in total adenine nucleotides, ATP, and P_i and a significant increase in AMP after fructose. These effects are less pronounced but still significant after sorbitol and even more pronounced after a mixture of glucose, fructose and xylitol. The other compounds tested caused small effects only.

If one considers these changes as a transient disturbance of homeostasis caused by rapid phosphorylation, the concentrations should level off in the course of continuous infusion. Experiments of this type are summarized in table 2. As can be seen from the data, the concentration of adenine nucleotides and of P_i had levelled off after 30 min and did not differ significantly from the controls in the course of 3 hrs. Using this technique there is no difference between fructose or the mixture of glucose, fructose and xylitol and the other compounds.

Discussion

The results of the experiments with rapid injection of carbohydrates confirm those of the Finnish authors (4). During continuous infusion, however, the steady state levels of adenine nucleotides and P_i in the liver did not differ significantly from controls, regardless of the type of carbohydrate used. Thus injection of a compound which is rapidly phosphorylated in the liver causes changes in metabolite levels. During prolonged infusion these changed levels soon approach the original steady state values. Despite normal stationary levels the turnover rate of these metabolites may be changed; this cannot be answered by the present investigation.

Table 1. Levels of adenine nucleotides and inorganic phosphate in rat livers after injection of carbohydrates and polyols

Substance injected	Number of rats	Metabolite levels (mmoles/g liver) ATP	ADP	AMP	Sum of adenine nucleotides	Pi
Tyrode's solution (control)	11	3,12 (0,51)	1,17 (0,11)	0,36 (0,15)	4,62 (0,32)	4,1 (0,44)
Fructose	11	1,04 (0,24)*	1,10 (0,12)	0,62 (0,20)*	2,77 (0,27)*	2,1 (0,37)*
Glucose	10	2,69 (0,35)	1,26 (0,12)	0,36 (0,08)	4,30 (0,23)	4,5 (0,63)
Xylitol	10	2,53 (0,32)	1,32 (0,15)	0,42 (0,10)	4,27 (0,25)	3,25 (0,42)*
Sorbitol	12	1,84 (0,53)	1,27 (0,14)	0,48 (0,12)	3,59 (0,52)*	2,9 (0,45)*
Glycerol	11	2,37 (0,38)	1,59 (0,14)	0,67 (0,14)	4,63 (0,55)	3,4 (0,66)
Fructose + Glucose + Xylitol	10	0,86 (0,15)*	1,19 (0,06)	0,67 (0,10)*	2,71 (0,24)*	2,0 (0,55)*
Xylitol + Sorbitol	10	2,28 (0,23)*	1,69 (0,04)*	0,69 (0,06)*	4,67 (0,22)	3,2 (0,56)*

Techniques see under "methods". Dosage: A total amount of 1 mmole; in mixtures: each component 0,3 mmole. Standard deviations in brackets. Asterisks indicate a significant difference with the control group at the 1 per cent level.

Table 2. Levels of adenine nucleotides and inorganic phosphate in rat livers after infusions of carbohydrates and polyols

Substance infused	Duration of infusion (min)	Metabolite levels (mmoles/g liver) ATP	ADP	AMP	Sum of adenine nucleotides	P_i
Tyrode's solution (control)	30	2,98 (0,31)	1,71 (0,27)	0,56 (0,15)	5,06 (0,51)	3,8 (0,49)
	60	2,81 (0,35)	1,98 (0,19)	0,69 (0,18)	5,48 (0,17)	4,1 (0,14)
	120	2,91 (0,31)	1,99 (0,27)	0,62 (0,15)	5,53 (0,44)	4,1 (0,19)
	180	2,63 (0,44)	1,76 (0,29)	0,55 (0,16)	4,93 (0,56)	5,0 (0,95)
Fructose	30	2,76 (0,36)	1,66 (0,27)	0,55 (0,14)	4,97 (0,38)	3,1 (0,71)
	60	2,50 (0,37)	1,66 (0,18)	0,58 (0,12)	4,73 (0,41)	3,7 (0,44)
	120	2,81 (0,24)	1,41 (0,12)	0,43 (0,07)	4,65 (0,31)	4,6 (0,16)
	180	2,30 (0,25)	1,40 (0,16)	0,44 (0,08)	4,14 (0,27)	4,3 (0,63)
Glucose	30	2,97 (0,56)	1,59 (0,25)	0,51 (0,15)	5,07 (0,33)	3,4 (0,32)
	60	3,02 (0,18)	1,60 (0,19)	0,46 (0,12)	5,08 (0,10)	3,6 (0,77)
	120	3,20 (0,32)	1,52 (0,24)	0,43 (0,11)	5,15 (0,57)	3,8 (0,28)
	180	2,31 (0,42)	1,41 (0,10)	0,48 (0,09)	4,20 (0,34)	4,5 (0,23)
Xylitol	30	2,71 (0,32)	1,68 (0,21)	0,61 (0,10)	4,99 (0,30)	3,8 (0,57)
	60	2,70 (0,46)	1,78 (0,10)	0,59 (0,12)	5,07 (0,34)	4,3 (0,67)
	120	2,80 (0,57)	1,57 (0,14)	0,54 (0,11)	4,91 (0,52)	4,8 (1,47)
	180	2,74 (0,50)	1,48 (0,16)	0,45 (0,09)	4,67 (0,51)	4,3 (0,08)
Sorbitol	30	2,66 (0,48)	1,75 (0,16)	0,55 (0,15)	4,96 (0,30)	3,3 (0,84)
	60	2,60 (0,37)	1,68 (0,26)	0,51 (0,13)	4,79 (0,48)	4,2 (0,49)
	120	2,81 (0,39)	1,68 (0,12)	0,57 (0,15)	5,06 (0,32)	5,2 (0,55)
	180	2,28 (0,50)	1,47 (0,19)	0,54 (0,17)	4,29 (0,33)	4,8 (1,16)
Glucose + Fructose + Xylitol	30	2,88 (0,19)	1,45 (0,29)	1,01 (0,04)	5,35 (0,33)	4,1 (0,61)

Each group consists of 10 rats. Techniques see under "methods". Infusion rate 1,5 mmoles · $kg^{-1} \cdot h^{-1}$. Standard deviations in brackets.

Our results with continuous infusion differ from those of *Woods* et al. (5) in liver perfusion experiments. The reason for this discrepancy is probably the extremely high fructose concentration used by these authors. The perfusion medium contained 10 mM (180 mg/100 ml) fructose. From our values for total clearance of fructose in rats, which is around 30 ml · min^{-1} · kg^{-1} (12) we can easily calculate that in order to reach such a blood level in vivo it would be necessary to infuse 3,24 g · kg^{-1} · h^{-1}.

This is a highly unrealistic dose. Even our dose of 1,5 g · kg^{-1} · h^{-1} exceeds the recommendable dose for humans which is around 0,5 g · kg^{-1} · h^{-1}. During the infusion of fructose in doses of 1,0 g · kg^{-1} · h^{-1} many people suffer from pain in the upper abdomen (7, 13).

Thus, if the recommendation is followed in parenteral nutrition not to supply carbohydrates other than glucose in doses exceeding 0,5 g · kg^{-1} · h^{-1}, changes in adenine nucleotide levels in the liver and disturbance of liver metabolism should not occur.

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Summary

Injection of large doses of fructose, sorbitol, or a mixture of glucose, fructose and xylitol in rats causes a drop of liver ATP, total adenine nucleotides and P_i and a rise of AMP, which is in agreement with data from the literature.

These changes are considered as a transient disturbance of homeostasis by compounds which are rapidly phosporylated in the liver. This is confirmed by the fact that during continuous infusion of these and other compounds at doses of 1,5 g · kg^{-1} · h^{-1} there was no such change. It is concluded that infusions of fructose or of the other carbohydrates tested with rates not exceeding those recommended for parenteral nutrition (0,5 g · kg^{-1} · h^{-1}) are not likely to disturb liver metabolism by changing levels of adenine nucleotides.

Zusammenfassung

In Übereinstimmung mit Angaben in der Literatur fanden wir nach Injektion hoher Dosen von Fructose, Sorbit oder einem Gemisch aus Glucose, Fructose und Xylit an Ratten einen Abfall von ATP, Gesamt-Adeninnucleotiden und anorganischem Phosphat in der Leber und einen Anstieg an AMP.

Wir halten diese Veränderungen für eine vorübergehende Störung der Homöostase durch Verbindungen, die in der Leber sehr rasch phosphoryliert werden. Tatsächlich findet man bei kontinuierlicher Infusion dieser Kohlenhydrate in Dosen von 1,5 g · kg^{-1} · $Std.^{-1}$ keine derartigen Veränderungen. Es ist anzunehmen, daß Fructose oder die anderen untersuchten Kohlenhydrate keine Störung des Leberstoffwechsels durch Absinken des Spiegels an Adeninnucleotiden verursachen können, wenn die Dosierung die für die parenterale Ernährung empfohlene Grenze von 0,5 g · kg^{-1} · $Std.^{-1}$ nicht überschreitet.

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