Feeding sugar overnight maintains metabolic homeostasis in rats and is preferable to overnight starvation

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Summary

Rats are often starved overnight for many different reasons. Overnight starvation causes loss of body and liver weights, depletion of liver glycogen, decrease of blood glucose and loss of amino acids because of gluconeogenesis. Providing pure sucrose cubes as the sole overnight nutrient is a simple, inexpensive way to empty the gastrointestinal (GI) tract, while minimizing liver changes and preventing decrease of blood glucose and loss of amino acids. Adding sugars to the overnight drinking water as the sole nutrient has the same beneficial effects, provided the type of sugar and its concentration allow for sufficient intake and provided hyponatremia is avoided. Feeding sucrose cubes or sugar solutions will empty the gastrointestinal tract as effectively as starvation. In all instances, simple precautions against coprophagy and pica should be taken in order to secure optimal benefit.

Keywords Blood glucose; blood urea; brief starvation; gastrointestinal tract; gluconeogenesis; liver glycogen; sucrose diet

Surgeons and anaesthesiologists are currently re-thinking the advisability of fasting patients the night before surgery (Fasting *et al.* 1998, American Society of Anesthesiologists 1999, Ferrari *et al.* 1999). Overnight fasts are not completely innocuous because even brief starvation may cause loss of body and liver weights, depletion of hepatic glycogen, decrease of blood glucose and increase of gluconeogenesis from amino acids (Nygren *et al.* 1995, Barton 1996). Movement of fluid to compensate for haemorrhage may be impaired. Thirst and hunger may contribute to anxiety. Experimentalists starve their animal subjects overnight before surgery in order to empty the GI tract. An empty GI tract facilitates gastric intubation, reduces accidental penetration of stomach or caecum by intraperitoneal injections, avoids interference by food with absorption or metabolism of drugs or nutrients under study, and provides basal conditions for blood chemistry. However, overnight starvation may be particularly detrimental to rats because they are normally nocturnal feeders. In the present work, we show that the unwanted effects of overnight starvation of rats can be eliminated or attenuated by feeding pure sugars in solid or solution forms, while still yielding an empty GI tract. (A preliminary report has appeared, Levine & Saltzman 1998.)

Materials and methods

Inbred Lewis rats of both sexes were raised from Harlan Sprague–Dawley stock under conventional conditions in hanging plastic shoe-box type cages with hardwood litter. Tap water and Laboratory Rodent Diet 5001

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(PMI Feeds, St Louis, Missouri, USA) were freely available. Fluorescent lighting from 06:00 to 18:00 h and 15 air changes/h were provided.

Each experiment started at 14:00 h and lasted only 20 or 44 h. The rats were housed in pairs. Raised metal grids were inserted on the floor of the cages and bedding material was omitted in order to reduce coprophagy (Levine & Saltzman 1999) and eliminate consumption of bedding ('pica'). The rats were weighed and assigned randomly to groups that were starved or fed either their usual Rodent Diet 5001 or pure sucrose cubes (Domino Dots, Domino Sugar Corp., New York, New York, USA). In other experiments, the usual diet (5001) was replaced by various sugars (40 g) dissolved in tap water to make a final volume of 400 ml, and dispensed in one pint glass bottles (18 × 6.5 cm). Loss by leakage was minimal. Rats that weighed 150–230 g are here designated 'small', and rats of 350–450 g are designated 'large'.

At 10:00 h of the next day, or the day after that, the rats were re-weighed, anaesthetized with carbon dioxide and bled from the inferior vena cava. The liver and the entire GI tract with contents (including the abdominal portion of the oesophagus) were removed and weighed immediately. A sample of the left hepatic lobe was fixed in Bouin's fluid, embedded in paraffin, sectioned and stained with haematoxylin–eosin and periodic acid–Schiff–haematoxylin. Serum was analysed for glucose, urea N, sodium and chloride in a Hitachi BMD model 736-30 analyser. Statistical analysis of results was performed by analysis of variance, as described previously (Levine & Saltzman 1998).

If feeding sucrose as the sole nutrient is to replace overnight starvation, it is important to answer one further question: would sucrose feeding interfere with the recovery process when feeding of the usual diet (5001) is resumed? Toward this end, groups of rats were starved or fed sucrose cubes for 44 h or fed their usual diet. Then *all* the rats were provided with the usual 5001 diet for 20 h, at which time necropsies were performed.

Results

The GI tract of control rats fed their usual diet had abundant content at all levels. It constituted almost 11% of body weight in small rats. GI tracts after 20 h of starvation or sucrose feeding had hardly any content and weighed much less (Table 1). After 44 h of either starvation or sucrose feeding, there was a small, further reduction in content so that the GI tracts were reduced to one-half the weight of GI tracts from rats fed the usual 5001 diet.

Liver weights were strikingly reduced by starvation for 20 or 44 h, but the weight loss was attenuated when sucrose was fed (Table 1). Serum glucose levels were reduced in starved rats but not in rats fed sucrose cubes for either 20 or 44 h. Serum urea levels were variably affected by starvation but strikingly reduced by sucrose feeding. Periodic acid–Schiff staining of the liver of starved rats was negative, indicating loss of hepatic glycogen, but staining was positive after sucrose feeding. In summary, either starvation or sucrose diet emptied the GI tract but sucrose feeding attenuated the atrophy of the liver and the loss of liver glycogen, reduced the serum

Table 1 Effects of feeding sucrose cubes as sole nutrient for 20 or 44 h on gastrointestinal (GI) tract, liver and serum of small rats

Diet	GI weight (% body)	Liver weight (% body)	Serum glucose (mg/dl)	Serum urea N (mg/dl)
Rodent diet 5001	10.94 ± 0.42	4.94 ± 0.03	172 ± 4	23.0 ± 1.0
Sucrose cubes 20 h	6.18 ± 0.35	4.25 ± 0.31	163 ± 1	6.5 ± 0.5
Sucrose cubes 44 h	5.36 ± 0.15	4.09 ± 0.22	193 ± 10	5.5 ± 0.5
No food 20 h	6.00 ± 0.28	3.28 ± 0.03	72 ± 2	14.5 ± 0.5
No food 44 h	5.29 ± 0.02	3.22 ± 0.11	136 ± 11	26.0 ± 1.0

Data are averages (± SD) of groups of four male rats that were 8 weeks old and weighed 200–230 g. Tap water was freely available. GI tract (with contents) and liver were weighed fresh

Table 2 Effects of feeding sucrose cubes as sole nutrient for 20 or 44 h on gastrointestinal (GI) tract, liver and serum of large rats

Diet	GI weight (% body)	Liver weight (% body)	Serum glucose (mg/dl)	Serum urea N (mg/dl)
Rodent diet 5001	8.74±1.07	4.21±0.29	178±19	22.5±1.0
Sucrose cubes 20 h	7.02±0.87	4.30±0.16	168±10	10.3±1.7
Sucrose cubes 44 h	4.58±0.34	4.00±0.22	179±3	7.3±1.3
No food 20 h	4.93±0.27	3.60±0.13	127±9	14.8±2.3
No food 44 h	4.52±0.06	3.26±0.04	100±2	14.0±1.0

Data are averages (±SD) of groups of four male rats that were 12 weeks old and weighed 350–450 g

urea, and maintained the normal serum glucose.

Starving or feeding sucrose to large rats yielded essentially the same results except that the GI tract was not completely emptied by 20 h of sucrose cube feeding (Table 2). These large rats had correspondingly large GI tracts which were completely emptied only after 44 h of sucrose diet (intermediate times were not studied).

Rats that had been subjected to starvation or sucrose cube feeding for 44 h and then given their usual 5001 diet consumed more food than the control rats that had never been deprived of their usual diet (Table 3). Both of these experimental groups had a complete reversal of the weight loss in GI tract and liver; in fact they had slightly heavier GI tracts and liver than the controls, probably related to the increased food consumption (Table 3). Clearly, prior sucrose feeding had not interfered with the return of the GI tract and liver to their normal or even supernormal weights after the weight loss caused by deprivation of food.

Sugar imbibed in the drinking water overnight produced results similar to those obtained by feeding sucrose cubes. These rats were not getting any solid food, so the GI tract was emptied in 20 h regardless of the type of sugar or its concentration (Table 4). Drinking glucose (10%) was also very effective in avoiding atrophy of the liver, but lower concentrations were ineffective. Maltose (10%) was moderately effective but, surprisingly, 10% sucrose did not prevent liver atrophy. This apparent anomaly was explained by measuring consumption of the drinking fluid. Glucose or maltose at 10% were relished by the rats who consumed approximately 125 to 150 ml/rat in 20 h. On the other hand, only about 75 ml/rat of 10% sucrose was imbibed (about 7.5 g sucrose). This was far below the consumption of sugar cubes, usually about 15 g/rat in 20 h. Also, fructose and mannose were not taken in sufficient amounts to be effective. Glucose at 5, 2.5 or 1% was ineffective because of low sugar content in addition to a much less intake. Some of these sugar preparations were able to maintain serum glucose levels and decrease serum urea levels even though they failed to prevent liver atrophy (Table 4). With large rats, glucose 10%, like sucrose cubes, was effective for preventing liver atrophy, but emptying of the GI tract was much better after 2 days of treatment, as in Table 2 (not included in tables).

In one additional experiment (not included in Table 4) 10% glucose or 10% maltose solutions as sole nutrients maintained serum glucose levels and decreased serum urea

Table 3 Recovery of gastrointestinal (GI) and liver weights in one day (20 h) from 2 days (44 h) of starvation or feeding sucrose cubes

Food		GI	Liver
Days 1,2	Day 3 (intake)	(% body)	(% body)
5001	5001 (11 g/rat)	10.31±0.30	4.08±0.06
Sucrose	5001 (19 g/rat)	12.45±0.03	4.36±0.05
None	5001 (38 g/rat)	10.99±0.41	4.39±0.04

Data are averages (±SD) of groups of four female rats that weighed 160–170 g. Food 5001 refers to Rodent Diet 5001. The rats were starved or fed 5001 or sucrose for 2 days, then all had access to 5001 for one further day (amount eaten in parentheses)

levels but failed to mitigate the loss of liver mass. In these rats, serum sodium levels were reduced to 129–139 mEq/l compared with 144–146 mEq/l in fasted rats and in control rats fed their usual diet in the same experiment. Chloride was also reduced to 86–99 mEq/l, compared with 101–105 mEq/l in the fasted rats and in the controls. No low serum sodium or chloride levels had occurred in all the experiments of Tables 1, 2 and 4.

In order to prevent a recurrence of hyponatremia, the anomalous experiment was repeated with 1% NaCl added to all the solutions. The 10% glucose/saline and 10% maltose/saline solutions were imbibed in even greater amounts (170–180 ml/rat in 20 h) than the corresponding sugars without added NaCl, and serum sodium and chloride levels remained normal (146–148 mEq/l and 105–108 mEq/l respectively). These salt-supplemented solutions protected the liver from starvation-induced atrophy (Table 5) as well as the salt-free solutions had done (Table 4).

Statistical analysis

ANOVA revealed that weights of the GI tract from rats that had been starved or fed sucrose cubes for one or 2 days were significantly different (less) than GI weights of control rats fed their usual 5001 diet ($P < 0.0001$). Hepatic weights of rats that had been starved for one or 2 days were significantly different (less) than hepatic weights of rats fed sucrose cubes or their usual 5001 diet ($P < 0.01$), but hepatic weights of sucrose-fed and 5001-fed rats were not significantly different from each other. Analysis of serum chemistry results showed that serum glucose of starved rats was significantly less ($P < 0.0001$) than glucose of rats fed sucrose cubes or their usual 5001 diet, and serum urea of rats fed sucrose cubes

Table 4 Effects of sugar solution as sole nutrient for 20 h on rats' gastrointestinal (GI) tract, liver and serum

Food	Drink	Consumption of sugar (g)	GI (% body)	Liver (% body)	Serum glucose (mg/dl)	Serum urea N (mg/dl)
5001	Water	0	10.48±0.58	4.37±0.10	155±33	20.2±4.3
None	Glucose 10%	13.8	6.57±0.29	4.23±0.33	165±17	6.2±0.8
None	Glucose 5%	3.7	6.28±0.59	3.42±0.16	147±13	8.5±0.5
None	Glucose 2.5%	0.4	6.21±0.46	3.33±0.03	152±21	8.5±0.5
None	Glucose 1%	0.2	6.00±0.11	3.58±0.01	101±6	16.0±1.0
None	Maltose 10%	11.4	6.61±0.47	3.92±0.05	214±14	6.0±1.5
None	Sucrose 10%	7.5	6.15±0.32	3.46±0.17	173±13	10.5±1.5
None	Water	0	6.05±0.39	3.53±0.12	102±11	15.7±2.7

Data are averages (±SD) of groups of four or six female rats that weighed 150–200 g. Food 5001 refers to Rodent Diet 5001. Consumption of sugar calculated from concentration and average intake volume

Table 5 Effects of salt-supplemented sugar solution as sole nutrient for 20 h on rats' gastrointestinal (GI) tract, liver and serum

Food	Drink	GI weight (% body)	Liver weight (% body)	Serum glucose (mg/dl)	Serum urea N (mg/dl)
5001	Saline	9.26±0.25	4.18±0.04	167±4	25.0±5.0
None	Glucose–saline	6.63±0.57	3.60±0.08	147±4	5.5±1.5
None	Maltose–saline	6.96±0.19	3.77±0.08	167±10	6.0±1.0
None	Sucrose–saline	6.77±0.34	3.31±0.21	175±13	9.5±2.5
None	Saline	5.73±0.58	3.22±0.16	90±2	16.0±1.0

Data are averages (±SD) of groups of four female rats that weighed 160–180 g. Sugar solutions were all 10% (w/v) concentration. Food 5001 refers to Rodent Diet 5001. Saline refers to 1.0% NaCl

was significantly less ($P < 0.0001$) than urea of rats starved or fed their usual 5001 diet.

Discussion

Glucose is an essential nutrient and urea is a nitrogenous waste product destined for excretion. Therefore, the maintenance of normal glucose levels in blood and a decrease of urea levels seem to be desirable. Similarly, avoiding (or reducing) loss of normal liver mass and glycogen content is desirable. Therefore, we advocate feeding sugar overnight rather than starving the rats for all indications, unless sugar is specifically contraindicated. It should be remembered that all the experiments reported herein involved the use of elevated metal grids and omission of bedding overnight in order to minimize coprophagy and pica, which could reduce the desired emptying of the GI tract. Also, the data show that more than 20 h may be needed for sucrose feeding to empty the GI tract completely in large rats.

With these reservations in mind, the use of sucrose cubes is a convenient and inexpensive expedient. However, it is likely that some investigators will prefer to provide needed sugar in the drinking water. The data show that this is quite practical if 10% glucose or maltose is used. Any other sugar in the drinking water, even sucrose, may not be consumed in sufficient amounts to maintain liver weight even though serum glucose is kept at a normal level and serum urea is reduced. Therefore, it is important to determine if sugar in the drinking water is consumed in sufficient volume and concentration to reap all the benefits described. Additions of NaCl as well as glucose to the drinking water are advisable to prevent hyponatremia, although we encountered this complication only once. Hyponatremia was never encountered when sucrose was fed as cubes. Polyuria caused by increased fluid intake may have depleted body sodium and chloride in these fasted rats, but the reason for the occurrence in this single experiment was not elucidated, nor was the nature of the interaction between hyponatremia and liver mass.

Loss of liver mass after starvation is caused partly by glycogenolysis, and lack of glycogen is followed by gluconeogenesis from amino acids in order to maintain blood glucose levels. Providing sucrose cubes or glucose solution reduces loss of glycogen and thereby reduces the need for gluconeogenesis from amino acids. Decreased utilization of amino acids preserves valuable proteins and results in decreased nitrogenous waste products, hence a lower blood urea (Jahoor & Wolfe 1987, Ljungqvist *et al.* 1990, Rothman *et al.* 1991, Neese *et al.* 1995, Wolf 1995, Landau *et al.* 1996, Jeukendrup *et al.* 1999). All these considerations favour replacement of brief starvation by brief periods of sucrose or glucose feeding.

The blood urea levels reported in the tables are more easily understood in the light of the dual origin of urea. In fed rats, urea is derived from nitrogenous components of the diet. In fasted rats, urea is derived during gluconeogenesis from nitrogen in endogenous proteins and amino acids. Rats fed only sugars (which contain no nitrogen and which prevent gluconeogenesis) have neither exogenous nor major endogenous sources of nitrogen, hence the very low urea levels.

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