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Effects of Fructose, Sucrose and Glucose Feeding on Plasma Insulin Concentrations and on Adipose-Tissue Clearing-Factor Lipase Activity in the Rat

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The rise in adipose-tissue clearing-factor lipase activity that results from feeding glucose to starved rats cannot be duplicated by giving equicaloric amounts of fructose or sucrose. An inability of the administered fructose and sucrose to raise the plasma insulin concentration probably accounts for this failure in enzyme response.

In man and in a variety of animal species dietary fructose has been shown to be converted in the liver into plasma triglycerides to a greater extent than dietary glucose (Nikkila, 1969; Topping & Mayes, 1973). On the basis of this evidence, it has been suggested that the high concentrations of plasma triglycerides which are induced by diets rich in sucrose may be due to enhanced hepatic conversion into triglyceride of the fructose component of the sucrose molecule and subsequent increased release of this triglyceride into the bloodstream (Macdonald, 1973).

The possibility that such a high plasma triglyceride concentration may also be contributed to by an impairment of the mechanism for removal of triglycerides from the circulation needs also to be considered, however. Such removal is believed to depend on the hydrolysis of the triglycerides in the capillary beds of the extrahepatic tissues by the enzyme clearing-factor lipase or lipoprotein lipase (Robinson, 1970). The activity of this enzyme in adipose tissue, which is a major site of uptake of the plasma triglycerides under conditions of caloric excess, declines on starvation as the uptake of plasma triglycerides by the tissue falls (Robinson, 1970). Whereas the administration of glucose to rats in the starved state causes the clearing-factor lipase activity of the tissue to increase over a period of a few hours (Pokrajac & Lossow, 1967; Otway *et al.*, 1971), and this rise can be correlated with a rise in the tissue's capacity for triglyceride uptake (Garfinkel *et al.*, 1967), Bar-on & Stein (1968) found that the administration of fructose caused no such increase in enzyme activity. Moreover, it has been briefly reported that the activity of the enzyme in adipose tissue of rats fed on diets rich in sucrose is lower than in the tissue of animals fed on diets rich in starch (Bruckdorfer *et al.*, 1972*a*).

These findings clearly raise the possibility that a low clearing-factor lipase activity in adipose tissue could contribute to the raised plasma triglyceride concentrations found in animals fed on sucrose. However, they are also of interest from another standpoint. Thus increases in the adipose-tissue clearing-factor lipase activity of starved rats occur when the tissue is incubated for a few hours *in vitro* in media containing either glucose or fructose (Wing *et al.*, 1966). Insulin is required in the incubation medium for optimal increases in enzyme activity under such conditions, and Bruckdorfer *et al.* (1972*b*) have shown that plasma insulin concentrations in rats fed on diets rich in sucrose or fructose for several weeks are much lower than those in animals fed on diets rich in starch or glucose. Thus the failure of fructose and sucrose administration to raise the adipose-tissue clearing-factor lipase activity of starved rats may be due to their inability to produce an adequate increase in the plasma insulin concentration. In the present study this possibility has been investigated by measuring simultaneously plasma insulin concentrations and adipose-tissue clearing-factor lipase activities 3h after the administration of glucose, fructose and sucrose to rats in the starved state.

Four groups of male albino rats of the Wistar strain that had been maintained on their normal laboratory diet (Oxoid pasteurized diet 41B; Herbert Styles Ltd., Bewdley, Worcs., U.K.) and that had body weights of between 190 and 200g were starved for 24h. Then, to the rats in three of the groups, 3ml of equicaloric solutions of glucose (100%, w/v), fructose or sucrose were administered by stomach tube between 9 and 9:30 a.m. and between 10:30 and 11 a.m., the rats in the remaining group being given 3ml of water at the same times. All the dosages were given with the rats under light ether anaesthesia and the sugars were all of AnalaR grade. The animals were allowed to recover from the anaesthesia between each manipulation and then, between 12 noon and 12:30 p.m., i.e. 3h after the first dosage, they were again anaesthetized with ether and killed by exsanguination from the abdominal aorta. The blood was collected into plastic tubes, which were kept at 4°C and which contained sufficient powdered heparin (Pularin; Evans Medical, Speke, Liverpool, U.K.) to prevent coagulation. While the collection of blood was proceeding, both the epididymal fat-bodies were removed. After they had been weighed, acetone–ether-dried preparations were made from them and the clearing-factor lipase

Table 1. *Effects of glucose, fructose and sucrose feeding on plasma constituents and on adipose-tissue clearing-factor lipase activity*

Four groups of six male rats were starved for 24h. The animals in three of the groups were given equicaloric solutions of glucose, fructose or sucrose by stomach tube between 9 and 9:30 a.m. and between 10:30 and 11 a.m. The animals in the fourth group were given 3ml of water at the same times. Between 12 noon and 12:30p.m. all the rats were killed by exsanguination and their epididymal fat-bodies were removed. Acetone–ether-dried preparations were made from these and their clearing-factor lipase activities were measured. Glucose, fructose, triglyceride, free fatty acid and immunoreactive insulin concentrations were also measured in the plasma separated from each blood sample. For each determination, duplicate portions of plasma were taken and duplicate assays were performed on each. The values given in the Table are the means (±S.D.) of the measurements carried out on the animals in each group. The numbers of animals in the groups are given in parentheses. The significance of the differences between the group values was estimated by using Behren's modification of Student's *t* test (Fisher & Yates, 1957). The mean (±S.D.) weight of the fat-bodies was 420±56mg and there were no significant differences among the different groups. Similar determinations were also carried out on rats fed *ad libitum*, and the values for these are also given in the Table.

	Plasma concentration of					Adipose-tissue clearing-factor lipase activity (units/g fresh wt. of tissue)
Substance administered	Glucose (mg/100ml)	Fructose (mg/100ml)	Triglyceride (μmol/100ml)	Free fatty acid (μmol/100ml)	Immunoreactive insulin (μunits/ml)	
Fructose	99±21 (14)	27±10 (8)	120±23 (14)	47± 8 (14)	29± 9 (18)	44±14 (20)
Glucose	89±10 (14)	—	55±32 (14)	22± 6 (14)	67±18 (19)	119±30 (19)
Sucrose	95±14 (14)	15± 4 (8)	121±35 (14)	36± 6 (14)	36±11 (18)	51±14 (20)
Water	52± 8 (14)	—	58±28 (14)	75±10 (14)	26±14 (17)	40±20 (20)
Animals fed *ad libitum*	127±11 (16)	—	103±26 (16)	28± 6 (8)	69±14 (16)	218±55 (16)

activities of these were measured in terms of their rate of hydrolysis of an appropriate triglyceride substrate as previously described (Cryer *et al.*, 1973). One unit of enzyme activity is defined as being that which releases 1μmol of free fatty acid/h at 37°C. After centrifugation of the blood, concentrations of glucose, fructose, triglyceride and free fatty acid in the plasma were determined by methods described by Bergmeyer & Bernt (1963), Roe (1934), Fletcher (1968) and Wing & Robinson (1968) respectively, and plasma immunoreactive insulin was measured by using the test combination supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Glucose, as well as fructose, standards were carried through the method for fructose determination and the plasma fructose values have been corrected for interference by plasma glucose.

In all, three experiments of the above design were carried out. The findings in each were essentially the same and the combined results are shown in Table 1, which also includes values for similar measurements made on plasma samples taken from rats fed *ad libitum* and killed between 8:30 and 9:30 a.m.

Plasma glucose concentrations were very similar in all the carbohydrate-fed groups at 3h after administration of the sugars and, though they were not as high as in the rats fed *ad libitum*, they were significantly ($P<0.001$) greater than in the control animals given water. Plasma free fatty acid concentrations were significantly ($P<0.001$) decreased in all the carbohydrate-fed groups at this time, and in the animals fed on glucose they were as low as in those fed *ad libitum*. Sucrose feeding caused significantly less depression of the free fatty acid concentration than did glucose feeding ($P<0.001$), and fructose feeding caused significantly less depression than did sucrose feeding ($P<0.001$).

Fructose was not measurable in the plasma of rats fed on glucose, nor was it detected in plasma from control animals given water or from animals fed *ad libitum*. It was present, however, in the plasma of rats fed on either fructose or sucrose, and in such animals the plasma triglyceride concentrations were also significantly raised ($P<0.001$) above those in the glucose-fed rats, which themselves were not significantly different from those in the control group given only water.

The above results were as expected from the results of previous studies (Bar-on & Stein, 1968; Bruckdorfer *et al.*, 1972*b*). However, of particular interest was the failure of both fructose and sucrose administration to raise either the adipose-tissue clearing-factor lipase activity or the plasma immunoreactive insulin concentration above the values found in the control animals given only water. Glucose administration, on the other hand, caused the plasma insulin concentration to rise to values characteristic of rats fed *ad libitum* and the adipose-tissue clearing-factor lipase activity to increase twofold ($P<0.001$) to approximately 50% of that found in animals fed *ad libitum*.

The overall positive correlation coefficient between the adipose-tissue clearing-factor lipase activity and the serum insulin concentration calculated from the

results in Table 1 is 0.77 ($n = 88$). This is highly significant ($P < 0.001$), and the present findings therefore strongly support the view, advanced on the basis of previous work (Robinson, 1970; Reichl, 1972; Borensztajn *et al.*, 1972), that insulin plays a major role in the control of the activity of the enzyme in adipose tissue. Moreover, they show that the plasma glucose concentration certainly cannot be the sole determinant of the activity of the adipose-tissue enzyme.

Adipose tissue is by no means the only important extrahepatic site of clearing-factor lipase activity. Nevertheless, the present findings clearly also raise the possibility that, because the feeding of fructose and sucrose to starved rats does not raise the activity of the enzyme in adipose tissue as does the feeding of glucose, these sugars may also fail to enhance the rate of triglyceride removal from the plasma. This, together with the effects that they have in increasing the rate of triglyceride entry into the plasma from the liver, could therefore be responsible for the hypertriglyceridaemia which their administration produces. Although Nikkila & Ojala (1966) found no significant differences in the rates of removal of triglycerides injected intravenously in rats fed on either fructose or glucose, they pointed out that their results did not exclude the possibility of a decrease in the rate of removal in the animals fed on fructose sufficient to account for the rise in the plasma triglyceride concentration. The question therefore seems to warrant further investigation.

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