

Effects of dietary glycerol on the serum glyceride level of men and women

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1. The serum glycerides of men were raised 1–2.5 h after the ingestion of glycerol, whereas in young women given glycerol the serum glycerides were not raised. In men who had a supplementation of glycerol to their free-choice diet there was a rise in the concentration of fasting serum glycerides, a rise that was not found when a similar supplementation was given to young women.
2. These findings would support the tentative suggestion that dietary fructose does not follow the Emden–Meyerhof pathway to the same extent as glucose. The increased serum glyceride levels reported after high-fructose diets may be due to an increased availability of the glycerol moiety of glycerides.

In man fructose-containing and glucose-containing carbohydrates have different effects on serum lipid levels (Macdonald & Braithwaite, 1964; Antar & Ohlson 1965; Grande, Anderson & Keys, 1965; Kuo & Bassett, 1965; Kaufmann, Poznanski, Blondheim & Stein, 1966; McGandy, Hegsted, Myers & Stare, 1966). This distinction is clear from a study of the effects of oral fructose and oral glucose on the glyceride concentration of the plasma (Macdonald, 1966). When a high proportion of the dietary carbohydrate is fructose the level of the glyceride fraction in fasting plasma is elevated, both in healthy men and in patients with hyperglyceridemia. This paper is concerned with testing a hypothesis which would explain the special relation of fructose to plasma glycerides.

Hypothesis

The fructose molecule makes a greater contribution than the glucose molecule towards the formation of glycerides in the liver (Nikkilä, 1966) and significantly more [¹⁴C]fructose was recovered in the glycerol moiety than in the fatty acids of liver and serum triglycerides of rats (Bar-On & Stein, 1968). An implication of these findings is that the ingestion of fructose results in a greater production of α -glycerol phosphate (glycerol-1-phosphate) than does the ingestion of glucose, and it is this increased α -glycerol phosphate that leads to the increased level of serum glycerides (Fig. 1).

Dietary fructose, before conversion into glucose, is split into dihydroxy-acetone-P, and glyceraldehyde and the latter is then phosphorylated to glyceraldehyde-P. The enzymes necessary for the conversion of fructose into glucose seem to occur both in the gut wall and liver (Salomon & Johnson, 1959). However, not all the fructose that is taken by mouth is immediately converted into glucose (Lamb, 1950), with the result that the concentration of fructose in the peripheral venous plasma, which is usually very low when fasting, can rise to levels of 10–15 mg/100 ml.

The presence of fructose in systemic venous blood after fructose ingestion means

that the rate of conversion of fructose into glucose is limited and that some fructose, at first, escapes this conversion. It has been suggested that not all the glyceraldehyde arising from fructose is metabolized by phosphorylation to triose phosphate (Ginsburg & Hers, 1960) and in man some may be converted, by alcohol dehydrogenase, into glycerol (Heinz, Lamprecht & Kirsch, 1968). This may be responsible for the rise in serum triglycerides seen after fructose but not after glucose ingestion.

For the hypothesis that fructose causes an increase in the α -glycerol phosphate pool to be tenable, any increase in this pool by substances other than fructose should lead to an increase in serum glyceride concentration.

The experiments described below were devised to test the hypothesis.

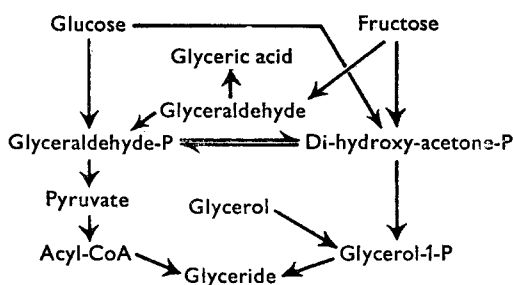


Fig. 1. Some of the pathways in glucose and fructose metabolism.

EXPERIMENTAL

(A) *The effect of acute ingestion of glycerol*

After a 12 h overnight fast a single dose of glycerol (1 ml/kg body-weight in 1 ml water/kg body-weight) was given by mouth to ten healthy men aged 22–45 years. The serum glyceride concentration was followed for 4 h after the ingestion of the glycerol. As controls the results were compared (1) with those following the ingestion, under similar conditions, of a volume of water similar to that of the glycerol test meal (i.e. 2 ml/kg body-weight) and (2) with those in which no fluid was taken. There was an interval of at least 7 d between each experiment in each individual. All subjects took the glycerol first, the water second, and only five volunteered for the experiment in which no fluid was taken.

In contrast to the findings in men, it has been reported that in young women the fasting serum glyceride level is not altered by a high intake of fructose or fructose-containing carbohydrate such as sucrose (Beveridge, Jagannathan & Connell, 1964; Klugh & Irwin, 1966; Macdonald, 1966). Post-menopausal women respond to dietary fructose in a manner similar to that found in men. In order to learn whether the influence of the sex hormones acted before or after the α -glycerol-phosphate stage a single dose of glycerol (1 ml/kg body-weight in 1 ml water/kg body-weight) was given to ten healthy women aged 18–35 years. As in the men, a volume of water similar to that of the glycerol was ingested by five of the women. The women did not do the experiment where no fluid was taken. The menstrual history was not elicited. The results were compared with those found in the men.

(B) The effect of chronic ingestion of glycerol

Twelve men aged 19–27 years were given 1 ml glycerol/kg body-weight per 24 h, divided into three doses. The subjects were told to eat as and when they wished and the glycerol could be taken with water or coffee. This regimen lasted for 42 d and after 21 d the number of volunteers decreased until the 6th week when there were five. Venous blood was taken weekly after a 12 h fast.

A similar experiment was carried out on eight women aged 20–26 years. All the volunteers were from the teaching or technical staff or students. During the acute and chronic experiments the subjects carried out their normal duties. It was not considered necessary to attempt to determine the previous dietary pattern of the subjects. The stage of the menstrual cycle during the experiments was known but the information was insufficient to show whether glycerol metabolism was affected by these hormonal fluctuations.

Chemical methods

The lipids from 1 ml of serum were extracted and, after weighing the total lipid, the glyceride fraction was separated by thin-layer chromatography, extracted and weighed (Macdonald, 1968).

RESULTS

(A) Acute ingestion

The mean and standard error of glyceride concentration for each time interval are seen in Table 1.

The level of glyceride for each individual was subtracted from his or her corresponding value at 0 time. This was done because of the relatively wide range of values found between the subjects. Comparisons between values were carried out by Student's *t* test, and findings were considered significant when $P < 0.025$.

Men

Glycerol meal. Compared with the fasting value, a significant increase in glycerides (mean = 15 mg/100 ml) was found at 1, 1.5 and 2 h after ingestion of the glycerol.

Water meal. The mean change from the fasting level of glycerides after the ingestion of the water meal showed that at all times, except 3.5 h, there was no significant fall.

Starvation. In the five men who continued to fast after arriving in the laboratory in the morning there was no change in the level of serum glyceride.

Women

Glycerol meal. No significant change was seen in the glyceride concentration.

Water meal. No significant change occurred in the glyceride level after a water meal.

*(B) Chronic ingestion**Weight*

The mean change in weight in the men during the 42 d chronic ingestion of glycerol was a gain of 0.4 kg (SE = ± 0.70) and in the women a fall of 0.1 kg (SE = ± 0.10).

Table 1. Mean (mg/100 ml) and standard error of the serum glyceride level at intervals after acute ingestion of glycerol or water and after starvation in men and young women

Subjects	0 h		0.5 h		1 h		1.5 h		2 h		2.5 h		3 h		3.5 h		4 h		
	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	
Men	104	12.4	110	15.7	126	11.6	118	10.8	113	13.1	114	10.0	106	11.1	119	11.4	105	13.0	
									Glycerol										
									Water										
Women	100	13.6	93	14.0	93	12.1	89	13.0	87	12.0	82	14.4	83	13.9	82	13.1	86	16.0	
									Starvation										
	107	9.0	113	11.6	101	13.5	102	13.9	101	11.3	97	11.1	102	8.1	108	11.0	111	4.8	
								Glycerol											
								Water											
	88	8.3	84	6.3	84	7.7	81	8.8	81	8.5	87	8.8	88	8.1	84	7.1	87	6.5	
									Water										
	87	16.6	87	16.7	92	17.0	94	16.5	89	18.7	97	20.3	88	16.4	84	19.2	78	10.0	

Glycerides (Table 2)

Men. The mean levels showed a tendency to rise and then return to the control level by 42 d.

The fasting serum of all the subjects was clear when the experiment was started but by 21 d three of the men had a cloudy fasting serum.

Women. The glyceride concentration tended to rise and then fall again.

An approach that can be used to assess and compare the response of the men and the women to chronic glycerol ingestion is to determine the mean increase in serum triglyceride concentration throughout the time on the glycerol supplementation. When this was done with the values for the men the mean increase was 20 mg/100 ml serum (SE = ± 4.1) and this mean overall increase was highly significant ($P < 0.001$). A similar procedure with the triglyceride values for the women showed an overall increase of 6 mg/100 ml serum (SE ± 3.2) and this value was not significant. When these mean overall increases are compared the value for the men is significantly greater than that for the women ($0.01 > P > 0.005$).

Table 2. Mean 7 d values (mg/100 ml) and standard errors for the fasting serum triglycerides after chronic ingestion of glycerol by human subjects for periods of 21–42 d

(The results have been divided into those for subjects who ingested the glycerol for 21 d, 28 d, 35 d and 42 d)

		7 d		14 d		21 d		28 d		35 d		42 d		No. of subjects
		0	1	2	3	4	5	6						
Men	\bar{x}	97	110	125	111	—	—	—	—	—	—	—	—	12
	SE	11.4	19.9	15.3	18.8	—	—	—	—	—	—	—	—	—
	\bar{x}	81	85	111	90	94	—	—	—	—	—	—	—	7
	SE	11.8	8.5	16.0	19.3	20.7	—	—	—	—	—	—	—	—
	\bar{x}	75	83	99	89	89	101	—	—	—	—	—	—	6
	SE	12.5	9.7	14.1	20.4	20.3	15.1	—	—	—	—	—	—	—
Women	\bar{x}	67	75	94	77	85	96	97	—	—	—	—	—	5
	SE	11.9	7.5	16.5	18.8	24.3	18.2	9.4	—	—	—	—	—	—
	\bar{x}	81	80	88	89	95	94	77	—	—	—	—	—	—
	SE	11.5	11.7	15.2	8.7	12.0	10.3	10.2	—	—	—	—	—	8
	\bar{x}	—	—	—	—	—	—	—	—	—	—	—	—	—
	SE	—	—	—	—	—	—	—	—	—	—	—	—	—

DISCUSSION

In the formation of glycerides in vivo it is generally assumed that carbohydrate follows the pathway via pyruvate, acetyl CoA, acyl-CoA to fatty acid (Lynen, 1967). If, however, there is a difference in the amount of glyceride formed between one carbohydrate and another, then other pathways need to be considered as well as the possibility that the two carbohydrates use the same pathway, but with quantitative differences (Zakim & Herman, 1968). One pathway that fructose could follow is the one that leads to α -glycerol phosphate, but before this suggestion becomes a hypothesis it must be shown that the presence of an excess of α -glycerol phosphate leads to an increase in the serum glycerides, implying thereby that excess of α -glycerol phosphate does not wholly enter the Emden–Meyerhof pathway. There is some evidence to support this in the findings of Galton (1966) who showed that, in the adipose tissue from obese patients, the activity of the mitochondrial enzyme which oxidizes glycerol

phosphate was markedly reduced. As the concentration of α -glycerol phosphate may be rate-limiting in the synthesis of triglycerides (Tzur, Tal & Shapiro, 1964) Galton suggested that the possible increase in glycerol phosphate in the obese may be responsible for the increased genesis of glyceride in the adipose tissue of obese patients.

One way which might be expected to increase the α -glycerol phosphate is to give glycerol, and the experiments described here show that, in men, glycerol administration does lead to an increase in serum glyceride concentration. This fact would be consistent with the hypothesis that an increase in fasting serum glycerides following fructose ingestion is due, in some measure, to a primary increase in the glycerol moiety. This fact is also consistent with the finding, in rats, that the incorporation of labelled acetate into lipid, by liver slices, was not greater in animals fed fructose, or sucrose compared with glucose (Zakim, Pardini, Herman & Sauberlich, 1967).

Other evidence to support the hypothesis that the increased serum glyceride concentration following fructose ingestion is due to a primary increase in the glycerol moiety is found mainly in experimental animals. The glyceride formed after giving [U - ^{14}C]fructose intraperitoneally to rats has over 90% of the label in the glycerol fraction (Kupke & Lamprecht, 1967). After giving radioactive carbohydrates by mouth to fasting rats significantly more counts are found in the glycerol moiety of liver and plasma triglyceride after [^{14}C]fructose (Y. Maruhama & I. Macdonald, in preparation). The incorporation of 44% of the label of [U - ^{14}C]glucose into the glycerol moiety is reported by Cahill, Leboeuf & Renold (1959) in *in vitro* experiments on the epididymal fat of the rat. They further showed that when stimulated by insulin the extent of the incorporation of the label in the glycerol fell. Similar findings were reported by Vaughan (1961). One possible explanation, therefore, for the different extent of incorporation of the ^{14}C from fructose compared with glucose could be that the latter is associated with a greater output of insulin and, because of this, both glycerol and fructose increase the serum glyceride level by an increase in the availability of the glycerol portion of glycerides.

If the increase in glyceride seen after ingesting fructose is due to the increased availability of the glycerol rather than the fatty acid moiety, then what are the possible metabolic differences between dietary fructose and glucose that would support this suggestion?

(1) The presence of fructose in the peripheral venous blood after ingestion implies that not all the fructose is converted into glucose by the gut wall. In baboons the portal vein concentration of fructose after a sucrose meal (2 g/kg body-weight) may rise to 100 mg/100 ml (Crossley & Macdonald, 1970). It is possible that with smaller doses of sucrose (or fructose) all the fructose is converted into glucose by the gut wall and the metabolic differences between fructose and glucose will not then arise.

(2) Before fructose is converted into D-glyceraldehyde-P it is, unlike glucose, split to glyceraldehyde. It has been demonstrated in rats that not all the D-glyceraldehyde from fructose is metabolized by phosphorylation to the triose phosphate (Ginsburg & Hers, 1960). The glyceraldehyde which is not phosphorylated can be metabolized in the liver via glyceric acid (Lamprecht & Heinz, 1958) to 2-phosphoglyceric acid (Ichihara & Greenberg, 1957) and thence to pyruvate. It has also been suggested

that glyceraldehyde may be reduced to glycerol (White & Landau, 1965) and that this is possible in man (Heinz *et al.* 1968). Therefore more of the triose from fructose than from glucose may escape the Emden–Meyerhof pathway and get converted into α -glycerol-phosphate. This could account for the increase in glyceride with its greater specific activity in the glycerol fraction.

The second question that was posed was whether the difference in the glyceride response to fructose between men and young women was due to direct or indirect hormonal interference before or after the α -glycerol-phosphate stage. The fact that ingested glycerol did not raise the serum glycerides in young women, whereas it did in men, suggests that a hormonal effect on the metabolic pathway of glycerol does exist. This finding of a sex difference in glycerol metabolism does not necessarily mean that the sex difference in the glyceride response to fructose is due to the sex hormones acting at the same metabolic level. It is possible that the sex hormones responsible for the different response to fructose could be acting on an earlier stage in the metabolism of fructose, implying thereby that the hormones responsible for this different metabolic behaviour act on at least two stages of fructose metabolism. However, the influence of sex hormones exists at or beyond the α -glycerol-phosphate stage and could also account for the different glyceride response to fructose between men and young women.

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